unlike previous reports in [illegible]
NEFA, and **triglycerides** were not altered by LPS. Nonetheless, as the period of feed deprivation progressed from 12 to 36 h, plasma NEFA and **triglycerides** increased ($P < .05$) and plasma glucose tended to decrease. We believe that immunological challenge induces cytokine synthesis and secretion in swine which, in turn, may induce **protein** catabolism.

L85 ANSWER 20 OF 53 MEDLINE DUPLICATE 6
97387044 Document Number: 97387044. Short-term oestrogen replacement therapy improves **insulin** resistance, **lipids** and fibrinolysis in postmenopausal women with NIDDM. Brussaard H E; Gevers Leuven J A; Frolich M; Kluft C; Krans H M. (Department of Endocrinology and Metabolic Diseases, University Hospital, Leiden, The Netherlands.) DIABETOLOGIA, (1997 Jul) 40 (7) 843-9. Journal code: E93. ISSN: 0012-186X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Oestrogen replacement therapy is associated with a decreased risk of cardiovascular disease in postmenopausal women. Patients with non-**insulin**-dependent diabetes mellitus (NIDDM) have an increased cardiovascular risk. However, oestrogen replacement therapy is only reluctantly prescribed for patients with NIDDM. In a double blind randomized placebo controlled trial we assessed the effect of **oral** 17 beta-estradiol during 6 weeks in 40 postmenopausal women with NIDDM. Glycated haemoglobin (HbA1c), **insulin** sensitivity, suppressibility of hepatic glucose production, lipoprotein profile and parameters of fibrinolysis were determined. The oestrogen treated group demonstrated a significant decrease of HbA1c and in the normotriglyceridaemic group a significantly increased suppression of hepatic glucose production by **insulin**. Whole body glucose uptake and concentrations of non-esterified **fatty acids** did not change. LDL-cholesterol- and apolipoprotein B levels decreased, and HDL-cholesterol, its subfraction HDL2-cholesterol and apolipotrotein A1 increased. The plasma **triglyceride** level remained similar in both groups. Both the concentration of plasminogen activator inhibitor-1 **antigen** and its active subfraction decreased. Tissue type plasminogen activator activity increased significantly only in the normotriglyceridaemic group. Oestrogen replacement therapy improves

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Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM

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Summary Oestrogen replacement therapy is associated with a decreased risk of cardiovascular disease in postmenopausal women. Patients with non-insulin-dependent diabetes mellitus (NIDDM) have an increased cardiovascular risk. However, oestrogen replacement therapy is only reluctantly prescribed for patients with NIDDM. In a double blind randomized placebo controlled trial we assessed the effect of oral 17 β-estradiol during 6 weeks in 40 postmenopausal women with NIDDM. Glycated haemoglobin (HbA_{1c}), insulin sensitivity, suppressibility of hepatic glucose production, lipoprotein profile and parameters of fibrinolysis were determined. The oestrogen treated group demonstrated a significant decrease of HbA_{1c} and in the normotriglyceridaemic group a significantly increased suppression of hepatic glucose production by insulin. Whole body glucose uptake and concentrations of non-esterified fatty acids did not change. LDL-cholesterol- and apolipoprotein B levels decreased, and HDL-cholesterol, its subfraction HDL_2-cholesterol and apolipotrotein A1 increased. The plasma triglyceride level remained similar in both groups. Both the concentration of plasminogen activator inhibitor-1 antigen and its active subfraction decreased. Tissue type plasminogen activator activity increased significantly only in the normotriglyceridaemic group. Oestrogen replacement therapy improves insulin sensitivity in liver, glycaemic control, lipoprotein profile and fibrinolysis in postmenopausal women with NIDDM. For a definite answer as to whether oestrogens can be more liberally used in NIDDM patients, long term studies including the effect of progestogens are necessary. [Diabetologia (1997) 40: 843–849]

Keywords Oestrogen therapy, non-insulin-dependent diabetes mellitus, glucose regulation, insulin sensitivity, hepatic glucose production, lipoprotein profiles, coagulation factors, fibrinolysis.

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Abbreviations: Apo A1 and apo B, Apolipoprotein A1 and B; ERT, oestrogen replacement therapy; FSH, follicle stimulating hormone; HDL-chol, high density cholesterol; HGP_1 and HGP_2, hepatic glucose production basal (first step) and second step; HGP_{suppr}, suppression of HGP from the first to the second step; LDL-chol, low-density cholesterol; LH, luteinising hormone; NEFA, non-esterified fatty acids; $NEFA_{suppr}$, percentage suppression of NEFA from the first to the second step; t-PA-ag, tissue type plasminogen activator antigen; PAI-1, plasminogen activator inhibitor; VLDL-TG, very low density lipoprotein triglycerides; $WBGU_2$, whole body glucose uptake in the second step of the clamp; WHR, waist hip ratio.

Overt non-insulin-dependent diabetes mellitus (NIDDM) is the end of a continuum in metabolic insulin resistance with decreasing compensation by insulin production from the beta cell [1]. Risk factors for ischaemic heart disease, the most importance cause of death and disability in elderly patients with diabetes, like carbohydrate intolerance or manifest diabetes, visceral obesity, hypertriglyceridaemia [2], low HDL-cholesterol content in plasma [3] and hypertension [2] are often clustered in a more or less complete profile of interdependent metabolic abnormalities, called the insulin resistance syndrome [4]. The balance between coagulation and fibrinolysis is intimately linked with this profile [5–7]. In patients with NIDDM the cardiovascular risk is increased 2

to 5 times when compared with non-diabetic persons [8]. When dyslipidaemia is also present the relative risk may further increase to 15 times [9].

Before the menopause cardiovascular events are less frequent in women than in men. This suggests a protective role for oestrogens. This apparent protection disappears in diabetes [10]. Oestrogen replacement therapy (ERT) in non-diabetic women after the menopause was found to be associated with lower fasting glucose [11] and with lower insulin concentrations [11, 12]. Godsland et al. [13] found no significant difference between users of ERT and non-users. They estimated insulin sensitivity by mathematical modelling of intravenous glucose tolerance tests. Lindheim et al. [14] using insulin tolerance tests found only a difference for low dose conjugated oestrogens. In experimental animal studies lowering of blood glucose induced by insulin was enhanced oestrogen therapy [15, 16]. Recently the Postmenopausal Estrogen/Progestin Interventions (PEPI)-trial [17], a placebo controlled double blind study in non-diabetic women, showed a significant decrease of fasting glucose during oral treatment with conjugated equine oestrogens with no change in insulin levels.

ERT was associated with a 50% reduction of the relative risk in cardiovascular morbidity and mortality in observational studies in non-diabetic women [18]. Selection bias may have caused overestimation of the apparent protective effect of ERT [19]. However, both the observation in animal studies that oestrogens stimulate reverse cholesterol transport with decreased subintimal cholesterol ester accumulation [20] and the direct vasorelaxing effect of oestrogens seen in humans [21] strongly suggest a beneficial effect. Moreover, during ERT the lipoprotein profile [22] and parameters of fibrinolysis [23] changed favourably.

In diabetic postmenopausal women ERT is only reluctantly advised [24]. The adverse effects of oral contraceptives on glucose tolerance in patients with NIDDM [25] may have contributed to the hesitation.

We studied the short term effects of ERT on glycaemic control and on insulin resistance using the euglycaemic hyperinsulinaemic clamp in relation with their effects on lipoprotein profile and fibrinolysis in patients with NIDDM with normal or elevated plasma triglyceride level (cut-off 2.0 mmol/l).

Subjects and methods

Patients. We recruited 40 postmenopausal women with NIDDM from outpatient clinics of the University Hospital Leiden or by advertisements and articles in newspapers. We considered NIDDM to be present if diabetes had been controlled sufficiently with diet and/or oral hypoglycaemic agents for more than 1 year or when detectable plasma C-peptide concentrations after glucagon stimulation were found. Women were considered to be postmenopausal when they had no menstrual cycle for more than 1 year. This was confirmed biochemically by a concentration of follicle stimulating hormone (FSH) in plasma over 20 U/l, higher concentrations of FSH than luteinising hormone (LH) and a concentration of oestradiol under 50 pmol/l. Exclusion criteria were: manifest coronary heart disease; liver or renal impairment; endocrine abnormalities other than NIDDM; hereditary diseases of lipid metabolism, plasma triglyceride levels over 10 mmol/l; oestrogen sensitive tumours or a history of thromboembolic diseases. Also excluded were patients who used metformin, diuretics, lipid lowering drugs, corticosteroids, anticonvulsant therapy or postmenopausal hormonal replacement therapy within the previous 3 months. The study was approved by the committee of medical ethics of the University Hospital Leiden and all women gave informed consent before their entry to the study.

Study protocol. We conducted a 6-week double blind placebo controlled trial of 2 mg micronized 17-β oestradiol (Novo-Nordisk, Zoeterwoude, The Netherlands) in 40 postmenopausal women with NIDDM, who received once daily either ERT or placebo orally. The patients were randomized into two groups of 20 subjects. Randomization was performed by a pharmacist, who was the only person knowing the code. FSH and LH were measured after closure of the trial in order not to break the code indirectly. We postulated that the effect of ERT would be similar in the normotriglyceridaemic group (NTG, TG $\leq$ 2 mmol/l; $n = 27$) and the hypertriglyceridaemic group (HTG, TG > 2 mmol/l; $n = 13$). In the NTG group 14 patients received oestrogen and 13 patients placebo. In the HTG group 6 patients were treated with oestrogen and 7 with placebo. The patients were asked to keep a food diary. Before and after 6 weeks of treatment parameters of insulin resistance, lipoprotein metabolism and fibrinolysis were assessed. After the study all patients were treated with 5 mg medroxyprogesterone acetate orally except those who had undergone a hysterectomy in the past.

Two patients from the oestrogen-group reported vaginal spotting, for which they were referred to the gynaecological department. They did not withdraw from the study.

Metabolic investigations. All patients were admitted to the metabolic ward the day before the clamp study. To achieve euglycaemia regular insulin was infused intravenously during the overnight fasting period. At 08.00 hours a cannula was inserted in the other forearm for blood sampling. The arm was placed in a thermoregulated plexiglass box (50–70 °C) to obtain arterialized blood. Before the start of the clamp blood samples were drawn for the assessment of fibrinolytic parameters and lipoprotein profile.

A sequential euglycaemic (5.4 mmol/l) hyperinsulinaemic three-step clamp was performed in order to measure whole body glucose uptake (WBGU) at various insulin perfusion rates [6]. To assess the hepatic glucose production rate (HGP) we administered tritiated glucose (3-^{3}H-glucose, specific activity 499.5 GBq/mmol, NET-331C; Dupont, Boston, Mass., USA). as a tracer by fixed infusion in a rate of 10 µCi/h for 6 h, preceded by a bolus of 15 µCi. During the clamp study plasma glucose was determined every 5 min (Glucose analyser; Beckman Instruments, Palo Alto, Calif., USA). The duration of each step of the clamp was 2 h. During the last 30 min of each step (the equilibrium phase) four blood samples were drawn to estimate the insulin concentration and the specific activity of glucose. The concentration of non-esterified fatty acid (NEFA) was determined in the last blood sample of each step. A dose response curve was obtained by infusion of regular insulin in the following order: 1) basal step: as much insulin as needed to maintain euglycaemia; 2) second step: 1.25 mU ·

$kg^{-1} \cdot min^{-1}$, preceded by a bolus of $12.5\ mU \cdot kg^{-1}$; and 3) third step: $10\ mU \cdot kg^{-1} \cdot min^{-1}$, preceded by a bolus of $100\ mU \cdot kg^{-1}$. During the last two steps 20% glucose was infused to maintain euglycaemia. Forty to sixty mmol potassium chloride was added to 1000 ml glucose 20% solution to prevent hypokalaemia.

In the first step of the clamp WBGU was equal to HGP. In the next two steps WBGU was calculated according to the isotope dilution technique, being the quotient between the infusion rate of tritiated glucose and the specific activity of glucose in plasma. The specific activity is the quotient between 3-^{3}H glucose in counts per minute and μmol glucose in the same aliquots. The specific activity of tritiated glucose was assessed after deproteinizing plasma with $ZnSO_4$ and $Ba(OH)_2$.

The HGP in the second and third step of the clamp was calculated by subtracting the mean glucose infusion rate during the last 30 min from the calculated rate as mentioned above. In the third step HGP was zero. HGP suppression (HGP_{suppr}) was calculated as $(HGP_1\text{-}HGP_2)/HGP_1$ (where HGP_1 denotes HGP in step 1 and HGP_2 denotes HGP in step 2 of the three step clamp). The mean glucose infusion rate during the steady state of second and the third step was considered to be the WBGU if the mean glucose infusion rate exceeded the quotient between the infusion rate of tritiated glucose and the specific activity of glucose. Otherwise the WBGU was assumed to be equal to the above mentioned quotient.

Percentage NEFA suppression ($NEFA_{suppr}$) has been calculated as $100 \times (NEFA_1\text{-}NEFA_2)/(NEFA_1\text{-}NEFA_3)$, $NEFA_1$, $NEFA_2$ and $NEFA_3$ being the NEFA concentrations of the steady-state phase of the first, second and third step of the clamp respectively.

Analytical procedures. NEFA were determined using the NEFAC test (Wako Chemicals GmbH, Neuss, Germany) and immunoreactive insulin and C-peptide concentration in plasma with a radioimmunoassay method (Medgenix, Brussels respectively, Biolab, Brussels, Belgium). Glycated haemoglobin (HbA_{1c}) was measured by HPLC (Bio-Rad, Richmond, Calif., USA). Lipoprotein profile was assessed by density gradient ultracentrifugation [26]. Plasma triglyceride concentration was determined with GPO-PAP triglyceride reagent (Boehringer Mannheim, Mannheim, FRG). Plasma glucagon concentration was measured by a specific pancreas glucagon radioimmunoassay (Daiichi, Tokyo, Japan). Apo A1 and Apo B were assessed by rate immunonephelometry using an automated Beckman array analyser (Beckman Instruments). Plasminogen activator inhibitor type 1 antigen (PAI-1-ag) was determined by an ELISA method and active PAI-1-ag by an immunoassay specific for the determination of active PAI-1 (Organon Teknika, Turnhout, Belgium).

Tissue type plasminogen activator antigen (t-PA-ag) was measured by immunoassay (Imulyse; Biopool, Umea, Sweden) [27] and activity (t-PA-act.) by a bioimmunoassay (Coatest BIA t-PA, Chromogenix, Mölndal, Sweden) [28]. The PAI-1-PA complex was measured using an immunoassay (Thrombonostika; Organon Teknika, Boxtel, The Netherlands). Blood samples for assessment of fibrinolysis were collected in Stabilyte tubes to prevent binding of t-PA to PAI-1 [29].

Statistical analysis. The adequacy of the randomization was assessed by comparing the two treatment groups at baseline using the Mann-Whitney U-Test. The same test was used to assess treatment group differences (tgd) in changes from baseline. Correlation analysis was performed with the Spearman rank correlation test. We used the SOLO 4.0 statistical program (J. Hintze; BMDP Statistical Software, Los Angeles, Calif., USA).

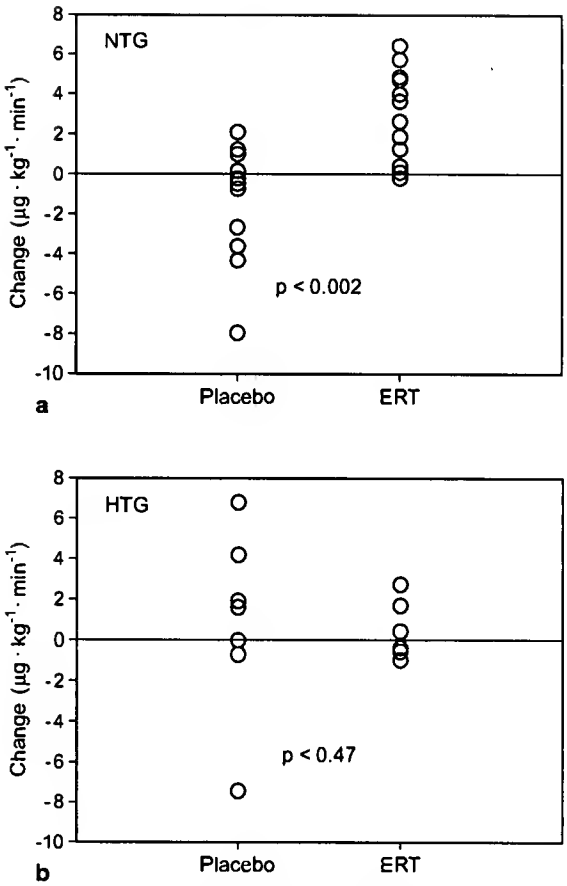

Fig. 1 a, b. Comparison of the effect of 6 weeks oestrogen treatment compared to placebo on hepatic glucose production suppression in an euglycaemic hyperinsulinaemic clamp in NIDDM patients with fasting normotriglyceridaemia (≤ 2.0 mmol/l; NTG) or hypertriglyceridaemia (> 2.0 mmol/l; HTG)

Results

The baseline values and the effects of treatment on insulin resistance, lipids, lipoprotein profile and fibrinolysis are summarized in Tables 1, 2 and 3 respectively. The two study groups did not significantly differ regarding age, BMI, WHR, duration of diabetes, HbA_{1c}, parameters of insulin resistance, parameters of lipoprotein profile and fibrinolysis.

Hypertension was found in 25% of the patients (i.e. systolic pressure > 170 and diastolic pressure > 90 mm Hg), 33% hypertriglyceridaemia (i.e. triglyceride concentration > 2 mmol/l) and 80% visceral obesity (i.e. WHR > 0.90). All patients had a creatinine clearance over 50 ml/min and microalbuminuria was less than 20 μg/min.

None of the patients dropped out of the study. Compliance was checked by measurement of plasma oestradiol levels at the second visit. These levels ranged from 106 to 670 pmol/l in the drug treated group and they were very low or not detectable in the placebo group. Blinding was maintained throughout the

Table 1. Demographics and parameters of insulin sensitivity at baseline and the change during therapy

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	17 β-oestradiol ($n = 20$)		
Age (years)	(a)	60.7 ± 5.2	60.4 ± 5.9	1.00	-
NIDDM (years)	(a)	8.9 ± 8.9	15 ± 13.3	0.12	-
Smoking *(n)*	(a)	3	4	-	-
BMI	(a)	28.4 ± 4.7	28.6 ± 6.1	0.99	0.04
(kg/m^2)	(b)	28.5 ± 4.6	29.0 ± 6.2		
WHR	(a)	0.96 ± 0.07	0.96 ± 0.07	0.80	0.73
	(b)	0.97 ± 0.06	0.98 ± 0.07		
HbA_{1c}	(a)	8.1 ± 1.6	8.7 ± 1.5	0.24	0.03
(%)	(b)	7.7 ± 1.4	8.1 ± 1.3		
C-peptide	(a)	0.54 ± 0.38	0.38 ± 0.39	0.11	0.26
(nmol/l)	(b)	0.52 ± 0.43	0.24 ± 0.22		
HGP_{suppr}	(a)	10.9 ± 3.3	9.4 ± 3.0	0.12	0.04
($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	(b)	10.4 ± 2.3	11.2 ± 3.5		
$WBGU_2$	(a)	21.8 ± 9.2	20.7 ± 15.2	0.19	0.87
($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	(b)	23.5 ± 10.8	22.1 ± 14.1		
$NEFA_{suppr}$	(a)	78.6 ± 30.5	82.8 ± 16.9	0.88	0.43
(%)	(b)	84.7 ± 8.1	78.9 ± 22.2		
Glucagon	(a)	166 ± 61.3	156 ± 62.9	0.47	0.18
(ng/l)	(b)	154 ± 60.9	151 ± 56.6		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

study. No significant group-difference was found in treatment related complaints, but in the end all patients guessed the right allocation of treatment group. The total energy intake and dietary composition in terms of protein, fat and carbohydrate content did not differ in the two treatment groups.

Insulin resistance (Table 1). The body mass index (BMI) increased by 1.5% during oestrogen treatment and by 0.7% during placebo (treatment group difference (tgd) $p = 0.04$). No difference in change of WHR was found between the two treatment groups.

The decrease in HbA_{1c} in the oestrogen treated group from 8.7 to 8.1% (mean change − 0.66 ± 0.67) was more than the decrease from 8.1 to 7.7% (mean change − 0.34 ± 0.45) in the placebo group (tgd $p < 0.03$). HGP_1 was not affected by ERT. However the change in HGP_{suppr} (1.77 ± 2.91 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was significantly greater in the oestrogen treated group than after placebo (0.51 ± 3.61 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (tgd $p < 0.04$) (Table 1). This was most pronounced in the NTG group (tgd $p < 0.002$, Fig. 1a). In the HTG group no significant difference in treatment effect was found (tgd $p = 0.47$, Fig. 1b). The HGP_{suppr} was significantly greater in the NTG than in the HTG group. The plasma level of glucagon, of C-peptide, the WBGU and the percentage of NEFA suppression changed in a comparable way in both groups, with no differences between the NTG and HTG groups. Before ERT, HbA_{1c} was not correlated with HGP or HGP_{suppr}. This was also true for the change in HbA_{1c} during ERT and the change in HGP and HGP_{suppr}.

Lipids, lipoproteins and apolipoproteins (Table 2). Plasma triglyceride levels did not change significantly in either of the two treatment groups, not even in the HTG patients. The same was true for VLDL-cholesterol. Plasma cholesterol concentration changed only slightly, while the LDL-cholesterol levels in the ERT group decreased by 14% compared to an increase of 2% in the control group (tgd $p = 0.0001$). Concomitantly, the plasma apo B levels decreased by 10% compared to an increase by 2% respectively (tgd $p = 0.001$). HDL-cholesterol increased by 22% compared to 3% (tgd $p < 0.0002$). This change in HDL-cholesterol level was mainly due to an increase in the less dense subfraction HDL_2 raising it by 49% compared to 5% (tgd $p < 0.0001$). No significant change was found in HDL_3-cholesterol concentration. Apo A1 was raised by 16% compared to 3% (tgd $p = 0.0001$). No effect in the mean density of the LPL peak or on the oxidisability of LDL [30] was seen.

Fibrinolysis (Table 3). Both total PAI-1 antigen and active PAI-1 antigen concentration decreased significantly during ERT compared with the placebo group (both tgd $p < 0.05$). The change in t-PA activity, t-PA antigen and in the PAI-t-PA complex concentration did not differ between treatment groups.

Table 2. Parameters of lipoprotein profile and the apolipoproteins at baseline and the effect of oestradiol or placebo on concentrations of plasma lipids and (apo)lipoproteins

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	Oestradiol ($n = 20$)		
Plasma cholesterol (mmol/l)	(a)	5.28 ± 0.66	5.25 ± 0.78	0.82	0.02
	(b)	5.32 ± 0.70	4.97 ± 0.70		
LDL-cholesterol (mmol/l)	(a)	3.36 ± 0.68	3.30 ± 0.74	0.68	0.0001
	(b)	3.42 ± 0.69	2.82 ± 0.67		
HDL-cholesterol (mmol/l)	(a)	1.20 ± 0.30	1.20 ± 0.47	0.88	0.0002
	(b)	1.24 ± 0.32	1.47 ± 0.56		
HDL_2-cholesterol (mmol/l)	(a)	0.36 ± 0.19	0.41 ± 0.29	0.89	0.0007
	(b)	0.38 ± 0.20	0.61 ± 0.39		
HDL_3-cholesterol (mmol/l)	(a)	0.84 ± 0.14	0.79 ± 0.21	0.47	0.14
	(b)	0.86 ± 0.17	0.85 ± 0.20		
VLDL-cholesterol (mmol/l)	(a)	0.64 ± 0.35	0.69 ± 0.43	0.86	0.61
	(b)	0.60 ± 0.42	0.62 ± 0.48		
Plasma triglyceride (mmol/l)	(a)	1.53 ± 0.83	1.74 ± 0.95	0.48	0.65
	(b)	1.61 ± 1.09	1.79 ± 1.09		
VLDL-triglyceride (mmol/l)	(a)	1.06 ± 0.63	1.09 ± 0.83	0.78	0.85
	(b)	1.07 ± 0.77	1.09 ± 0.84		
Apo A1 (g/l)	(a)	1.44 ± 0.18	1.39 ± 0.28	0.68	0.0001
	(b)	1.48 ± 0.18	1.61 ± 0.30		
Apo B (g/l)	(a)	1.27 ± 0.28	1.26 ± 0.36	0.89	0.0004
	(b)	1.30 ± 0.32	1.13 ± 0.29		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

Table 3. Parameters of fibrinolysis and the effect of oestradiol or placebo on parameters of fibrinolysis

		Baseline values (a) After therapy (b)		$p_{baseline}$	p_{change}
		Placebo ($n = 20$)	Oestradiol ($n = 20$)		
Active PA1-1 (ng/ml)	(a)	22.9 ± 25.3	17.5 ± 14.1	0.67	0.01
	(b)	25.5 ± 23.5	8.9 ± 5.9		
PA1-1 antigen (ng/ml)	(a)	129 ± 124	100 ± 63	0.83	0.03
	(b)	131 ± 118	72 ± 44		
tPA activity (IU/ml)	(a)	0.23 ± 0.14	0.26 ± 0.16	0.53	0.07
	(b)	0.21 ± 0.13	0.29 ± 0.15		
tPA antigen (ng/ml)	(a)	6.7 ± 2.7	6.7 ± 3.3	0.88	0.17
	(b)	6.7 ± 3.1	5.6 ± 2.7		
PA1-tPA complex (ng/ml)	(a)	12.0 ± 5.7	11.4 ± 6.5	0.68	0.40
	(b)	11.9 ± 5.4	10.3 ± 5.3		

Values are mean ± SD. The p-values indicate the probability that the mean baseline values ($p_{baseline}$) or the change in the two treatment groups (p_{change}) are identical

Discussion

The results of this study demonstrate for the first time that during short term oral ERT the control of NIDDM improves, as indicated by a decrease in HbA_{1c}. Our study indicates that among the three main sites of insulin resistance, the liver, skeletal muscle and adipose tissue [1], the liver (increased HGP suppression) is the major site of improvement of diabetic control during short term ERT. Inhibition of glucagon induced glycogenolysis, as has been shown in ovariectomized mice [16] or an inhibition of alpha-cell function, causing a fall of glucagon secretion [15] may be involved. We were not aware of the plasma glucagon levels in the portal blood in our patients, but the peripheral glucagon levels and their changes were similar in the two treatment groups.

We measured basal HGP after normalizing blood glucose values during the night preceeding the clamp to obtain comparable measurements. HGP is influenced by the blood glucose level, which was sometimes over 10 mmol/l on the evening preceding the study.

We found that the HGP suppression by insulin was not affected by ERT in HTG patients, while the suppression was strongly enhanced in NTG patients (tgd $p < 0.002$). It may be assumed that the increase of triglyceride synthesis during ERT [31] decreases the availability of NEFA for beta-oxidation. Beta-oxidation provides "fuel" for gluconeogenesis, but is rapidly saturated. It may be that in HTG patients due to the increased levels of NEFA, beta-oxidation is already saturated by high substrate availability so that eventual changes in substrate availability are not reflected in changed gluconeogenesis whereas in NTG patients the lower NEFA concentrations allow changes to become visible. An effect of ERT on intracellular availability of NEFA in mitochondria is not known.

Another source of fatty acids for the liver are core remnants of triglyceride rich lipoproteins and HDL_2 particles after they have been attacked by hepatic lipase. Oral administration of oestrogens inhibits hepatic lipase activity [32]. Thus, a reduced NEFA influx from this source may also have contributed to the improved HGP_{suppr} especially in NTG patients. Although the BMI was significantly increased in the ERT group as compared to the placebo group the WHR did not change. The WHR is a measure of abdominal obesity. An increased NEFA release from abdominal fat is important in the insulin resistance syndrome [2]. This is supported by our baseline data, i.e. WHR ($r_s = -0.49$, $p < 0.002$) and BMI ($r_s = -0.45$ $p < 0.005$) were negatively correlated with HGP_{suppr}.

Insulin resistance can also be caused by reduced blood flow in skeletal muscle and reduced capillary density [1, 33, 34]. However, we could not demonstrate an increase in WBGU during ERT. The effect of oestrogens on the re-esterification of NEFA with glycerol phosphate in adipose tissue, a site of insulin resistance [1], is unknown.

The change in plasma lipids and lipoproteins from baseline is in accordance with earlier reports on non-diabetic postmenopausal women [22]. The decrease of cholesterol in plasma is a reflection of the lowering of LDL-cholesterol. This was paralleled by a decrease in apo B concentration and may be explained by an increased LDL-cholesterol and apo B catabolism in liver, probably due to an increased number of apo LDL recetpors during ERT [31]. The effects on HDL-cholesterol were mainly due to an increase of its less dense, variable part, the HDL_2 subfraction. This may be explained by suppression of hepatic lipase activity by ERT [31]. Moreover, the increase in HDL was accompanied by a significant increase of apo A1 concentration probably by increased apo AI synthesis in the liver [35]. Although oestrogens enhance triglyceride synthesis in the liver, producing large "fluffy-puffy" VLDL particles [22], we did not find an increase in plasma VLDL triglyceride, or in VLDL-cholesterol concentration.

A high PAI-1 concentration and activity are associated with insulin resistance [5–7]. The lowering fits in the pattern of other changes observed during ERT, but the mechanism is not clear. Kroon et al. [23] demonstrated that ERT can induce a decrease in the plasma concentration of PAI-1 in non-diabetic postmenopausal women. During treatment with low-dose oral contraceptives a 50% decrease in PAI-1 has been observed [36]. We found a decrease in both active and total PAI-1 levels in postmenopausal women with NIDDM, suggesting an improvement of fibrinolysis.

Oestrogen replacement therapy is increasingly prescribed in postmenopausal women for various reasons. We studied the risk factors influenced by unopposed oestrogens. However, in practice combined therapy with progestogens is prescribed to minimize oestrogen-induced risk of endometrial carcinoma in women with an intact uterus. The effect of progestogens remains to be established. In diabetic women ERT is more reluctantly used [24] because of the presumed negative effects of oestrogens on carbohydrate and lipid metabolism [25]. A long term study on the effects of hormone replacement therapy among postmenopausal women with NIDDM should be performed before a more liberal policy is advisable.

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References

1. DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM, a balanced overview. Diabetes Care 15: 318–368
2. Kaplan NM (1989) The Deadly Quartet, Upper-body obesity, glucose intolerance, hypertriglyceridemia and hypertension. Arch Intern Med 149: 1514–1520
3. Bierman EL (1992) Atherogenesis in diabetes. Arterioscler Thromb 12: 647–656
4. Reaven GM (1991) Resistance to insulin-stimulated glucose uptake and hyperinsulinemia: role in non-insulin dependent diabetes, high blood pressure, dyslipidaemia HTG and coronary heart disease. Diab Metab 17: 78–86
5. Juhan-Vague I, Thompson SG, Jespersen J (1993) Involvement of the hemostatic system in the insulin resistance syndrome, a study of 1500 patients with angina pectoris. Arterioscl Thromb 13: 1865–1873
6. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE (1993) The cardiovascular risk factor

plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism 42: 945–949
7. Brussaard HE, Gevers Leuven JA, Krans HMJ, Meijer P, Buytenhek R, Kluft C (1994) Non-esterified fatty acids are related with hypofibrinolysis in type 2 diabetes mellitus. Fibrinolysis 8: 25–27
8. DeStefano F, Ford EA, Newman J et al. (1993) Risk factors for coronary heart disease mortality among persons with diabetes. Ann Epidemiol 3: 27–34
9. Assmann G, Schulte H (1989) Diabetes mellitus and hypertension in the elderly: concomitant hyperlipidemia and coronary heart disease risk. Am J Cardiol 63: 33H–37H
10. Barrett-Connor E (1994) Heart disease in women. Fert Ster 62: 127S–132S
11. Nabulsi AA, Folsom AR, White A et al. (1993) For the atherosclerosis risk in communities study investigators. Association of hormone replacement therapy with various cardiovascular risk factors in postmenopausal women. N Engl J Med 328: 1069–1075
12. Ferrara A, Barrett-Connor E, Wingard DL, Edelstein SL (1995) Sex differences in insulin levels in older adults and the effect of body size, oestrogen replacement therapy, and glucose tolerance status. The Rancho Bernardo Study. Diabetes Care 18: 220–225
13. Godsland IF, Gangar K, Walton C et al. (1993) Insulin resistance, secretion and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism 42: 846–853
14. Lindheim SR, Presser SC, Ditkoff EC, Vijod MA, Stanczyk FZ, Lobo RA (1993) A possible bimodel effect of oestrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. Fertil Steril 60: 664–667
15. Mandour T, Kissebah AH, Wynn V (1977) Mechanisms of oestrogen and progesterone effects on lipid and carbohydrate metabolism: alteration in the insulin : glucagon molar ratio and hepatic enzyme activity. Eur J Clin Invest 7: 181–187
16. Ahmed-Sorour H, Baily CJ (1980) Role of ovarian hormones in the long-term control of glucose homeostasis. Interaction wiht insulin, glucagon and epinephrine. Hormone Res 13: 396–403
17. The writing group for the PEPI trial (1995) Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA 273: 199–208
18. Stampfer MJ, Colditz GA, Willett WC et al. (1991) Postmenopausal oestrogen therapy and cardiovascular disease. Ten year follow-up from the nurses' health study. N Engl J Med 325: 756–762
19. Vandenbroucke JP (1995) How much of the cardioprotective effect of postmenopausal estrogens is real? Epidemiology 6: 207–208
20. Adams MR, Kaplan JR, Manuck SB, Koritnik DR, Parks JS, Wolfe MS (1990) Inhibition of coronary artery atherosclerosis by 17 β-oestradiol in ovariectomized monkeys. Lack of an effect of adding progesterone. Arteriosclerosis 10: 1051–1057
21. Collins P, Rosano GMC, Sarrel PM et al. (1995) 17 β-Estradiol attenuates acetylcholine-induced coronary artery constriction in women but not in men with coronary artery disease. Circulation 92: 24–30
22. Gevers Leuven JA (1994) Sex steroids and lipoprotein metabolism. Pharmac Ther 64: 99–126
23. Kroon UB, Silfverstolpe G, Tengborn L (1994) The effects of transdermal oestradiol and oral conjugated oestrogens on haemostasis variables. Thromb Haem 71: 420–423
24. Hemminki E, Sihvo S (1993) A review of postmenopausal hormone therapy recommendations: a potential for selection bias. Obstet Gynecol 82: 1021–1028
25. Godsland IL, Crook D, Simpson R et al. (1990) The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med 323: 1375–1381
26. Gevers Leuven JA, Mooren MV van der, Buytenhek R (1995) The effect of medrogestone on lipids and lipoproteins in postmenopausal women using conjugated oestrogens: an open randomized comparative study. Fertil Steril 64: 525–531
27. Ranby M, Bergsdorf N, Nilsson T, Mellbring G, Winblad B, Bucht G (1986) Age dependence of tissue plasminogen activator concentrations in plasma as studied by an improved enzyme linked immuno-sorbent. Clin Chem 32: 2160–2165
28. Meijer P, Boon R, Jie AFH, Rosen S, Kluft C (1992) Bioimmunoassay for tissue-type plasminogen activator (t-PA) in human plasma: evaluation of blood sampling and handling procedures and comparison with other t-PA activity methods. Fibrinolysis 6: 97–99
29. Ranby M, Sundell IB, Nilsson ThJJ (1989) Blood collection in strong acidic citrate anticoagulants used in a study of dietary influence and basal tPA activity. Thromb Haemost 62: 617–622
30. Brussaard HE, Gevers Leuven AJ, Kluft C, Krans HMJ, Duyvenvoorde W van, Huytenhek R, Laarse A van der, Princen HMG (1997) Effect of 17 β-estradiol on plasma lipids and LDL oxidation in postmenopausal women with type II diabetes mellitus. Arterioscler Throm Vasc Biol 17: 324–330
31. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM (1991) Effects of postmenopausal oestrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325: 1196–1204
32. Westerveld HT, Kock-Herman LAW, Rijn JM van, Erkelens DW, Bruin TWA de (1995) 17-beta estradiol improves postprandial lipid metabolism in postmenopausal women. J Clin Endocrin Metab 80: 249–253
33. Krotkiewski M, Seidell JC, Bjorntorp P (1990) Glucose tolerance and hyperinsulinaemia in obese women: role of adipose tissue distribution, muscle fibre characteristics and androgens. J Intern Med 228: 385–392
34. DelPrato S, Bonadonna RC, Bonora E et al. (1993) Characterization of cellular defects of insulin action in type 2 (non-insulin-dependent) diabetes mellitus. J Clin Invest 91: 484–494
35. Walsh BW, Li H, Sacks FM (1994) Effects of postmenopausal hormone replacement with oral and transdermal oestrogen on high density lipoprotein metabolism. J Lip Res 35: 2083–2093
36. Gevers Leuven JA, Kluft C, Dersjant-Roorda MC, Harthoorn-Lasthuizen EJ, Boer R de, Helmerhorst FM (1995) Effects of low-dose ethinylestradiol oral contraceptives differing in progestogenic compound on coagulation and fibrinolytic risk variables for venous and arterial thromboembolic diseases. In: Glas-Greenwalt P (ed.) Fibrinolysis in disease: molecular and hemovascular aspects of fibrinolysis. CRC Press, Boca-Raton, pp 226–234

and gamma-glutamyl transferase (gamma GT, I, IGF-I and T3 significantly while growth hormone decreased non-significantly. When saline was infused alone, G, TG, PL, Ca, AP, gamma GT, I, IGF-I and T3 decreased, while FFA, urea and sodium increased, but, changes of G, urea, AP, IGF-I and T3 were less marked than after **injection** of E. Potassium, total **protein** and albumin concentrations, and glutamyl dehydrogenase and glutamate oxalacetate transaminase activities were not significantly affected by either treatment. In conclusion, metabolic and endocrine changes during saline infusion alone were typical for food withdrawal. Changes of variables after administration of E were transient, biphasic or sustained, thus expressing complex interactions between metabolic parameters, endocrine factors and cytokines.

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Metformin and metoprolol CR treatment in non-obese men

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Abstract. Landin K, Tengborn L, Smith U (Department of Medicine, Sahlgrenska Hospital, University of Göteborg, Göteborg, Sweden). Metformin and metoprolol CR treatment in non-obese men. *Journal of Internal Medicine* 1994; 235: 335–341.

Objective. To study the effect of metformin and metoprolol CR on insulin sensitivity, blood lipids, fibrinolytic activity and blood pressure.

Design. A double-blind, placebo controlled, triple cross-over study with randomization to either metformin, 850 mg b.i.d., or metoprolol CR 100 mg o.d., or placebo for a period of 18 weeks. The glucose uptake was measured with the euglycaemic clamp technique after every 6 weeks' treatment period. Blood pressure and blood samples were taken every 3rd week.

Subjects. Eighteen non-obese men (53 ± 6 years of age).

Results. Metformin decreased C-peptide ($P < 0.02$), FFA ($P < 0.003$), total and low-density lipoprotein cholesterol, tissue plasminogen activator antigen and the urinary potassium excretion ($P < 0.05$ for all), but not blood pressure compared to placebo. Metoprolol CR reduced diastolic blood pressure and pulse rate; fasting free fatty acids and the urinary potassium increased ($P < 0.05$ for all). No effect of metformin or metoprolol CR was seen on the glucose disposal rate, blood glucose, plasma insulin, triglycerides, high-density lipoprotein cholesterol, lipoprotein(a), uric acid or plasminogen activator inhibitor 1 activity or antigen. The glucose uptake was not particularly decreased in these subjects.

Conclusion. The study shows that metformin has some favourable effects on metabolism and that metoprolol CR is fairly neutral in this regard. The lack of effect of metformin on glucose disposal rate and blood pressure can be explained by the fact that the individuals studied were neither insulin resistant nor hypertensive. The data does not preclude an antihypertensive effect by treating a concomitant insulin resistance.

Keywords: blood pressure, cholesterol, insulin, metformin, metoprolol, plasminogen activator inhibitor 1, triglycerides.

Introduction

Insulin resistance is associated with essential hypertension, whether or not treated and independent of obesity [1–4]. In a previous pilot study it was shown that specifically ameliorating insulin resistance with the biguanide metformin in non-diabetic, non-obese and previously untreated hypertensive subjects increased peripheral glucose uptake and reduced insulin levels, blood lipids, as well as the blood pressure [5]. These results support the important role of insulin resistance in hypertension as treatment with conventional antihypertensive agents, diuretics and beta blockers, do not lead to improvements in other associated risk factors apart from the blood pressure *per se* [6–8].

This study was carried out to investigate the effect of metformin and metoprolol CR in non-obese men with a history of hypertension. Effects on glucose uptake, blood lipids, fibrinolytic factors and blood pressure were studied.

Patients and methods

Patients

Eighteen men, mean age 53 ± 6 years with hypertension since 8.2 ± 7 years, were recruited by means of an advertisement in the local newspaper. Seven were treated with a beta blocker only, one with a calcium-antagonist only, and two with diuretics only. One patient took both a beta blocker

and a calcium antagonist, three took a beta blocker and a diuretic drug and four were untreated. Blood pressure at entry was 147 ± 8 mmHg systolic and 89 ± 6 mmHg diastolic during the mentioned treatment; the body mass index was 25.8 ± 2.0 kg m^{-2}. The waist/hip ratio was 0.95 ± 0.07. The study was approved by the Ethical Committee of the University of Göteborg and all men gave their informed consent.

Study design

The study was performed for 18 weeks and was of a double-blind, triple cross-over, placebo-controlled design as shown in Fig. 1. The subjects were randomized after their usual antihypertensive agents were withdrawn. Each treatment period lasted for 6 weeks and was followed by a euglycaemic clamp, collection of blood and urine samples, measurements of blood pressure and body weight as well as a physical examination. Blood samples and blood pressure were followed every 3rd week. Metformin (Glucophage®; Lipha Pharmaceuticals Ltd, Middlesex, UK) was given at a dose of 850 mg b.i.d. and metoprolol CR (Seloken ZOC®; Astra Hässle, Mölndal, Sweden) 100 mg once daily.

Anthropometry

Body height and weight were measured in the morning with the subjects wearing only underclothes. The waist circumference was measured with a soft tape midway between the lowest rib margin and the iliac crest in the standing position. The hip circumference was measured over the widest part of the gluteal region and the waist over hip circumference ratio (WHR) calculated.

Total body potassium was measured in a whole-body counter (Nuclear Enterprise Ltd, Edinburgh, UK) detecting naturally occurring ^{40}K. Lean body mass (LBM) was calculated with Forbes' formula [9] according to which 1 kg lean body mass contains 68.1 mmol potassium. Body fat was calculated by subtracting the lean body mass from the total body weight.

Biochemical analyses

Venous blood samples were drawn from an antecubital vein after an overnight fast. Glucose was determined using a glucose-6-phosphate dehydrogenase method (Beckman Instruments, Fullerton, CA, USA). Insulin was assayed with the Phadeseph Insulin RIA (Pharmacia, Uppsala, Sweden) and C-peptide by a radioimmunoassay method using commercially available reagents (Sangtec Medical, Dietzenbach, Germany). Cholesterol, triglycerides and lactate were determined enzymatically (Boehringer, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was analysed according to Seigler *et al.* [10] and the low-density lipoprotein (LDL) cholesterol calculated according to Friedewald's formula [11] where LDL cholesterol (mmol l^{-1}) = total cholesterol − HDL cholesterol − $0.45 \times$ triglycerides. Plasma free fatty acids (FFA) were determined with kits from WAKO Chemicals (Neuss, Germany). Uric acid was analysed with a standard method at the hospital's central laboratory. Lipoprotein(a) (Lp[a]) was determined using kits from Biopool, (Umeå, Sweden).

Glucose clamp. A hyperinsulinaemic, euglycaemic glucose clamp was performed to estimate insulin action (sensitivity) essentially as described by DeFronzo *et al.* [12] and as reported previously [13]. The insulin infusion (Actrapid® Human; Novo, Copenhagen, Denmark) rate was 0.08 IU kg^{-1} body weight h^{-1} which gave a mean plasma insulin level of 118 ± 32 mU l^{-1}. Glucose (20% weight/volume) was infused and the blood glucose levels kept at 5.2 ± 0.6 mmol l^{-1} throughout the clamp. The clamp lasted for 2 h and the glucose disposal was calculated from the steady-state glucose infusion rate during the last 30 min. The coefficient of variation (CV) was 5.0% for the repeatedly drawn (every 10 min) blood glucose samples during the clamp. The glucose disposal was expressed as mmol kg^{-1} body weight and per kg lean body mass per min.

Fibrinolytic process. Blood samples were drawn in precooled 5-ml Stabilyte vacutainer tubes (Biopool) containing 0.5 ml of 0.45 M sodium citrate buffer pH 4.3 (t-PA activity) and 5-ml vacutainer tubes containing 0.5 ml of 0.13 M trisodium citrate (Becton Dickinson, Meylan-Cedex, France) to the remaining assays. The tubes were immediately centrifuged at 4°C, 2000 *g* for 20 min. The plasma was aliquoted, snap frozen and stored at −80°C until analyses were performed.

Tissue plasminogen activator (t-PA) activity and antigen were assayed using Coa-Set t-PA and Coaliza t-PA kits, respectively (Chromogenix, Mölndal, Sweden). Plasminogen activator inhibition (PAI-1)

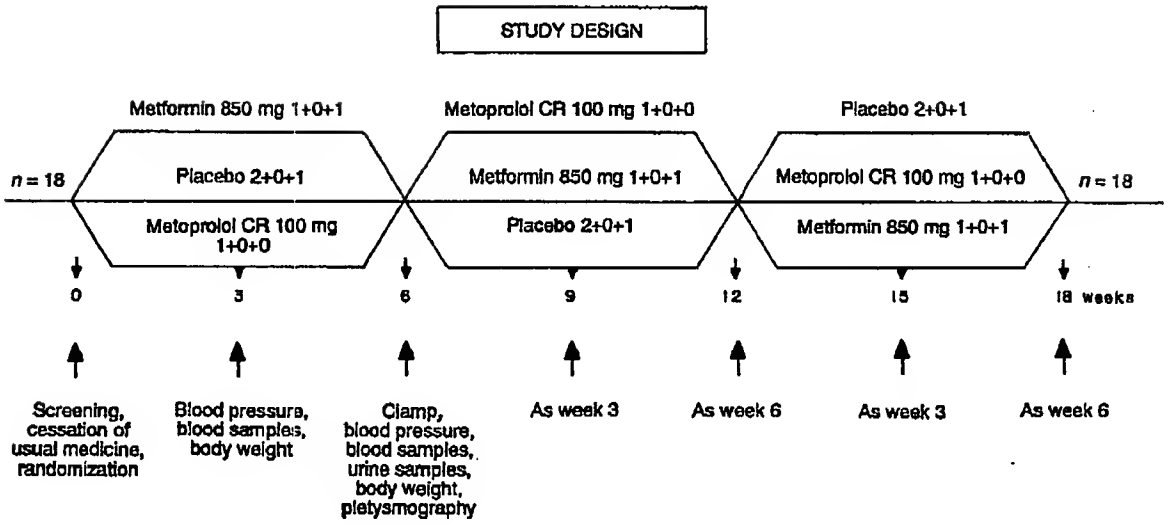

Fig. 1 Study design.

activity and antigen were determined using Spectrolyse(pL)PAI and TintElize PAI-1 kits, respectively (Biopool).

Fibrinogen was measured using a syneresis method [14]. All the tubes were immediately centrifuged at 4°C for 20 min at 2000 *g*. The CV was 10% for PAI-1 activity equal to 6 U ml^{-1} and 6% for PAI-1 activity equal to 44 U ml^{-1}. CV was 10% for the t-PA activity and antigen equal to 5 ng ml^{-1}, and 7% for t-PA antigen equal to 50 ng ml^{-1}.

Urinalyses. Urine was collected for 24 h to analyse cortisol, adrenaline and noradrenaline excretion. The hormones were determined with a fluorometer using an adsorption technique with aluminiumoxide [15]. Urine was also collected for 24 h for sodium, potassium and creatinine excretion and determined with a flame photometer (FLM 3; Radiometer, Copenhagen, Denmark).

Blood pressure measurements. The blood pressure shown is the mean of three measurements on the left arm after 10 min in the supine position. Diastolic pressure was measured as Korotkoff phase V. A random zero sphygmomanometer (Hawksley & Sons, Lancing, UK) was used with a cuff size corresponding to the circumference of the left arm. Resting pulse rate was registered simultaneously.

Blood flow measurements. Blood flow in both limbs were measured with a venous-occlusion pletysmograph (Periquant 3800; Gutmann-Medizinelektronik, Eurasburg, Germany) every 6 weeks before the clamp study.

Statistics

Mean values, standard deviations and other descriptive statistics were calculated for all variables. Analysis of variance with factors for patient, treatment, period and carry-over effects was used to compare each active treatment period with the placebo period. Least square means and 95% confidence intervals for the differences were calculated with corresponding *P*-values. A $P < 0.05$ was considered significant.

Results

During metformin treatment C-peptide levels ($P < 0.02$), FFA ($P < 0.003$), total cholesterol ($P < 0.02$) LDL, t-PA antigen and urinary potassium excretion ($P < 0.05$ for all). However, glucose disposal rate, blood pressure, triglycerides, HDL cholesterol, Lp(a), plasma insulin, fibrinogen, t-PA activity, PAI-1, uric acid, lactate, peripheral blood flow, pulse rate, body weight, urinary excretion of sodium, cortisol, catecholamines and creatinine remained unaltered on metformin treatment as compared to placebo (Tables 1 & 2).

Table 1 Effects of metformin and metoprolol CR vs. placebo on glucose and lipid metabolism in non-obese men ($n = 18$)

	Placebo	Metformin	P-level	Metoprolol CR	P-level
Blood glucose (mmol l^{-1})	5.1 (4.9–5.2)	4.9 (4.7–5.0)	NS	5.1 (4.9–5.2)	NS
Plasma insulin (mU l^{-1})	10 (8–11)	9 (8–10)	NS	9 (8–11)	NS
C-peptide (nmol l^{-1})	0.81 (0.74–0.90)	0.67 (0.61–0.75)	< 0.02	0.78 (0.79–0.86)	NS
Glucose disposal (mg kg^{-1} BW min^{-1})	6.7 (5.9–7.6)	6.1 (5.4–6.9)	NS	5.8 (5.1–6.5)	NS
Glucose disposal (mg kg^{-1} LBM min^{-1})	10.0 (9.1–10.9)	9.0 (8.5–9.6)	NS	8.9 (7.6–9.5)	NS
FFA (mmol l^{-1})	860 (697–885)	690 (556–735)	< 0.003	970 (865–1011)	< 0.02
Cholesterol (mmol l^{-1})	5.4 (5.3–5.6)	5.2 (5.0–5.3)	< 0.02	5.3 (5.2–5.5)	NS
LDL cholesterol (mmol l^{-1})	3.4 (3.3–3.6)	3.2 (3.0–3.3)	< 0.05	3.3 (3.1–3.5)	NS
HDL cholesterol (mmol l^{-1})	1.2 (1.1–1.3)	1.3 (1.2–1.3)	NS	1.1 (1.1–1.2)	NS
Lp(a) (mg l^{-1})	38.7 (25.9–57.6)	46.7 (31.4–69.7)	NS	57.4 (38.3–86.2)	NS
Triglycerides (mmol l^{-1})	1.5 (1.3–1.7)	1.4 (1.2–1.6)	NS	1.7 (1.5–1.9)	NS

P-levels vs. placebo. Least square means with 95% confidence intervals in parentheses.
BW, body weight; FFA, free fatty acids; HDL, high-density lipoprotein; LBM, lean body mass; LDL, low-density lipoprotein; Lp(a), lipoprotein (a).

Table 2 Effects of metformin and metoprolol CR vs. placebo on urinary excretion of potassium, blood flow in right (R) and left (L) limbs and other haemodynamic and fibrinolytic variables in non-obese men ($n = 18$)

	Placebo	Metformin	P-level	Metoprolol CR	P-level
U-potassium (mmol 24 h^{-1})	50 (41–52)	46 (41–53)	< 0.04	56 (50–63)	< 0.04
Blood flow (R) (ml 100 ml^{-1} min^{-1})	2.1 (1.5–2.9)	1.8 (1.3–2.5)	NS	2.0 (1.4–2.8)	NS
Blood flow (L) (ml 100 ml^{-1} min^{-1})	2.2 (1.7–2.8)	1.9 (1.5–2.4)	NS	1.8 (1.4–2.3)	NS
Heart rate (beats min^{-1})	61 (58–63)	62 (60–65)	NS	56 (54–59)	< 0.04
SBP (mmHg)	133 (125–137)	127 (120–131)	NS	129 (124–135)	NS
DBP (mmHg)	89 (87–92)	89 (85–89)	NS	85 (84–88)	< 0.05
PAI-1 activity (U ml^{-1})	14.0 (10.8–18.1)	10.9 (8.4–14.0)	NS	14.8 (11.5–19.1)	NS
PAI-1 antigen (ng ml^{-1})	19.1 (14.3–25.6)	12.8 (9.5–17.1)	NS	23.1 (17.1–31.1)	NS
t-PA activity (IU ml^{-1})	0.38 (0.29–0.50)	0.39 (0.30–0.51)	NS	0.36 (0.27–0.47)	NS
t-PA antigen (ng ml^{-1})	8.4 (7.7–9.1)	7.3 (6.7–7.9)	< 0.02	8.8 (8.1–9.5)	NS
Fibrinogen (g l^{-1})	2.9 (2.7–3.1)	2.6 (2.4–2.9)	NS	2.7 (2.5–3.0)	NS

P-levels vs. placebo. Least square means and 95% confidence intervals in parentheses.

Metoprolol CR decreased diastolic blood pressure and pulse rate ($P < 0.05$ for both), increased the levels of FFA and urinary potassium excretion ($P < 0.05$ for both). No significant changes were seen in glucose uptake, cholesterol, LDL and HDL cholesterol, Lp(a), triglycerides, insulin, C-peptide, fibrinolytic variables, blood flow or body weight (Tables 1 & 2). Uric acid, blood lactate and excretion of sodium, adrenaline, noradrenaline and cortisol were also unaffected by treatment.

The mean arterial blood pressure was negatively correlated to the glucose disposal rate ($r = -0.43$; $P < 0.05$) during the placebo period despite the rather normal insulin sensitivity. Glucose disposal rate was positively correlated to mean blood flow in the limbs ($r = 0.44$; $P < 0.05$) based on data from the placebo period (Fig. 2).

When comparing metformin with metoprolol CR, significantly lower levels of blood glucose, plasma insulin, triglycerides, t-PA antigen, PAI-1 antigen and higher HDL-cholesterol levels were seen during the metformin period (Tables 1 & 2).

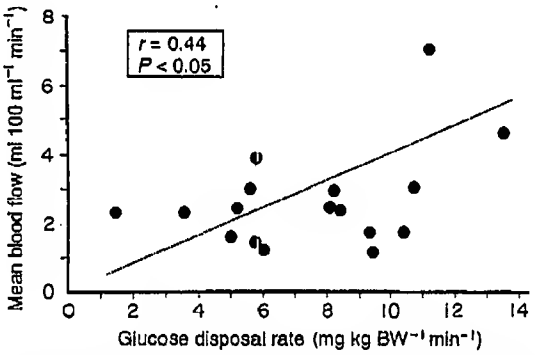

Fig. 2 Relation between glucose disposal rate and mean blood flow in the limbs during the placebo treatment period (n = 17); one subject is missing due to a technical error in the pletysmograph.

A tablet count indicated a good compliance of all participants. One patient suffered from vomiting during the initial 3 days of the placebo period and one patient had softer stools during the first week of metformin treatment.

Discussion

The positive effect of metformin on glucose uptake and blood pressure in our previous pilot study [5] could not be reproduced in these men with a history of essential hypertension. However, the men in the present study selected on the basis of having an established and treated hypertension were neither clearly insulin resistant nor did they have high blood pressure during the placebo or the other treatment periods. Their glucose uptake was about twice that of the weight-matched men in our previous study [5]. The glucose uptake increased by 21% during metformin treatment in that study. Hence, the initial normal insulin sensitivity might be an explanation for the lack of effect of metformin on the glucose disposal rate and, thereby, also its inability to lower the blood pressure as seen in the present study. In addition, although the hypertensive individuals were selected on the basis of chronic antihypertensive therapy, the low blood pressure seen during the placebo period raises the question of whether they were all truly hypertensive. Physical activity, body composition and the calculated insulin effect during the clamp were similar and cannot explain the differences in insulin sensitivity. Fasting plasma insulin did not differ but C-peptide was even higher in these men compared to those in the pilot study [5]. The blood lipid levels in all men of the present study were normal and lower than in the men of the pilot study [5]. Similar Lp(a) levels in both studies exclude factors linked to the Lp(a) gene as an explanation of the metabolic differences.

The lower C-peptide levels seen during the metformin period in the absence of an increased insulin sensitivity might mirror a direct effect on hepatic blood flow and clearance. A lipid-lowering effect of metformin without effects on glucose levels has also been reported earlier [16–17]. One explanation for this might be a direct effect of metformin on lipid synthesis in the liver; Another explanation is that metformin may have influenced insulin action on some metabolic variables but not on others. It is well known that, for instance, the antilipolytic effect is extremely sensitive to insulin, whilst glucose uptake requires higher insulin concentrations. Reduced free fatty acid levels in the face of unaltered plasma insulin as seen in these non-obese, non-diabetic men may be a reflection of this phenomenon. A direct effect of metformin on lipid oxidation is also possible [18].

Blood flow was found to be reduced by metformin treatment in a previous study of patients with peripheral artery disease [16]. However, no effect was seen in the present study of men with a normal peripheral circulation. Glucose disposal rate correlated to blood flow in the present study and this has also been reported previously in obese subjects [19]. Furthermore, glucose disposal correlated to blood pressure. An effect of metformin on blood flow might thus have played a role in the finding of our previous study that the blood pressure was reduced [5]. Metoprolol CR did not influence the blood flow in the limbs nor was glucose disposal rate changed.

Treatment with metoprolol CR caused few negative metabolic effects in the present study. Increased FFA levels, indicating an increased lipolysis and/or impaired eliminations by metoprolol, has been reported previously [20]. It is notable that 11 men had beta-blocker treatment for 8 years before the randomization and still had normal cholesterol and triglyceride levels. Previous studies have reported a further impairment of glucose and lipid metabolism as well as insulin sensitivity in hypertensive individuals treated with beta-blocking agents [6–8]. However, these studies included several overweight subjects who were insulin resistant even before treatment was started [6–8]. The men in the present study had similar mean body mass indexes to the average, age-matched healthy men in Scandinavia [21]. Their glucose and lipid levels were within the range defined as not pathologically elevated and, hence, did not exhibit the characteristics of a 'metabolic syndrome' [22].

Fibrinogen and PAI-1 activity were higher in the present men than in normotensive individuals [4] and similar to those in weight-matched, untreated hypertensives [4–5]. The fibrinolytic activity was not affected either by metformin or metoprolol CR in the present study. In an earlier study, metoprolol was found to decrease PAI-1 activity, even within the normal range [23].

In a recent study it was found that PAI-1 regulation is genotype-specific [24]. Lp(a) is a genetic marker and an independent risk factor for

cardiovascular disease [25]. Lp(a) also correlated to PAI-1 activity in the present study during placebo treatment ($r = 0.54$; $P < 0.01$; $n = 18$). Metformin did not affect Lp(a) levels in the pilot study (217 ± 147 vs. 200 ± 141 mg l^{-1}; $P > 0.05$) despite markedly improving glucose and lipid metabolism. Also in the present study, no significant change in PAI-1 activity was found, which might be explained by both the unaltered Lp(a), with its plasminogen structure homology (26), and the unchanged insulin levels. Significantly lower insulin, triglycerides, PAI-1 antigen and t-PA antigen levels but an unchanged glucose disposal rate were seen during metformin treatment when compared to metoprolol CR in the present study. Thus, in more pronounced hyperinsulinaemic and hypertensive subjects metformin, rather than metoprolol, could possibly exert beneficial effects on fibrinolysis. Metformin decreased both PAI-1 activity and insulin levels in obese subjects [27].

The potassium-sparing effect of metformin can be an effect of a decreased sodium absorption in the distal tubuli. This mechanism is thought to be modulated by insulin [28].

In conclusion, the present study shows that all individuals treated for hypertension are not insulin resistant or hyperinsulinaemic which is in accordance with a recent publication [29]. However, a correlation between blood pressure and glucose disposal rate was still found in the present study. The lack of effect of metformin on glucose uptake and blood pressure might be explained by both the normal insulin sensitivity and the fairly normal blood pressure in these individuals even during the placebo period. Furthermore, treatment with metoprolol CR only produced slight metabolic side-effects in these insulin-sensitive hypertensives. Hence, a potentially important role of insulin resistance in hypertension in some individuals still persists and specifically treating insulin resistance in these cases might be beneficial for blood flow, blood pressure as well as several other important risk factors for cardiovascular disease.

Acknowledgements

This study was supported by Astra Hässle AB (Mölndal, Sweden) and the Göteborg Medical Society. We thank O. K. Andersson MD, G. Holm MD, Carola Gustafsson, Marie-Louise Norberg and Kaisa Torstensson for excellent technical assistance.

References

1 Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S. Insulin resistance in essential hypertension. *N Engl Med* 1987; 317: 350–57.

2 Shen DC, Shieh SM, Fuh MMT, Wu DA, Chen YDI, Reaven GM. Resistance to insulin-stimulated-glucose uptake in patients with hypertension. *J Clin Endocrinol Metab* 1988; 66: 580–83.

3 Pollare T, Lithell H, Berne C. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. *Metabolism* 1990; 39: 167–74.

4 Landin K, Tengborn L, Smith U. Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. *J Intern Med* 1990; 227: 273–8.

5 Landin K, Tengborn L, Smith U. Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. *J Intern Med* 1991; 229: 181–7.

6 Bengtsson C, Blohmé G, Lapidus L. Do antihypertensive drugs precipitate diabetes? *Br Med J* 1984; 289: 1495–7.

7 Pollare T, Lithell H, Selinus I, Berne C. Sensitivity to insulin during treatment with atenolol and metoprolol: a randomized, double blind study of effects on carbohydrate and lipoprotein metabolism in hypertensive patients. *Br Med J* 1989; 298: 1152–7.

8 Lithell HOL. Effect of antihypertensive drugs on insulin, glucose and lipid metabolism. *Diabetes Care* 1991; 14: 203–9.

9 Forbes GB, Gallup J, Hursh JB. Estimation of total body fat from potassium-40 content. *Science* 1961; 133: 101–102.

10 Seigler, L, Wu WT. Separation of serum high-density lipoprotein for cholesterol determination: ultracentrifugation vs. precipitation with sodium phosphotungstate and magnesium chloride. *Clin Chem* 1981; 27: 838–41.

11 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 1: 499–502.

12 DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; 237: E214–23.

13 Landin K, Lönnroth P, Krotkiewski M, Holm G, Smith U. Increased insulin resistance and fat cell lipolysis in obese but not lean women with a high waist/hip ratio. *Eur J Clin Invest* 1990; 20: 530–35.

14 Nilsson IM, Olow B. Determination of fibrinogen and fibrinogenolytic activity. *Thromb Diathes Haemorrh* 1962; 8: 297–310.

15 von Euler US, Lishajko F. The estimation of catecholamines in urine. *Acta Physiol Scand* 1959; 45: 122–32.

16 Sirtori CR, Franceschini G, Gianfrenceschi G, Sirtori M, Montanari G, Bosisio E, Mantero E, Bondioli A. Metformin improves peripheral vascular flow in nonhyperlipidemic patients with arterial disease. *J Card Pharmacol* 1984; 6: 914–23.

17 Rains SGH, Wilson GA, Richmond W, Elkeles RS. The reduction of low density lipoprotein cholesterol by metformin is maintained with long-term therapy. *J Roy Soc Med* 1989; 82: 93–4.

18 Sparre Hermann L, Melander A. Biguanides: basic aspects and clinical uses. In: Alberti KGMM, DeFronzo RA, Zimmet P, eds.

International Textbook of Diabetes Mellitus. New York: John Wiley & Sons Ltd. 1992: 773–95.

19 Laakso M, Edelman SV, Brechtel G, Beron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese men. A novel mechanism for insulin resistance. *J Clin Invest* 1990; 85: 1844–52.

20 Wahrenberg H, Arner P, Engfeldt P, Haglund K, Rössner S, Östman J. Long-term β_1-selective adrenergic blockade and adrenergic receptors in human subcutaneous adipocytes. *Acta Med Scand* 1985; 217: 539–46.

21 Waaler HT. Height, weight and mortality. The Norwegian experience. *Acta Med Scand* 1984; 215 (Suppl. 679): 1–56.

22 Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988; 37: 1595–607.

23 Teger-Nilsson A-C, Dahlöf C, Eeglund E, Hedman C, Olsson G, Åblad B. Influence of metoprolol CR/ZOC on plasminogen activator inhibitor (PAI-1) in man: a pilot study. *J Clin Pharmacol* 1990; 30 (Suppl.): S132–7.

24 Dawson S, Hamsten A, Wiman B, Henney A, Humphries S. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. *Arterioscler Thromb* 1991; 11: 183–90.

25 Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein(a) and coronary heart disease: a prospective case-control study in a general population sample of middle-aged men. *Br Med J* 1990; 301: 1248–51.

26 McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987; 330: 132–7.

27 Vague P, Juhan-Vague I, Alessi MC, Badier C, Valadier J. Metformin decreases the high plasminogen activator inhibition capacity, plasma insulin and triglyceride levels in non-diabetic obese subjects. *Thromb Haemostas* 1987; 57: 326–8.

28 DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest* 1975; 55: 845–55.

29 Muller DC, Elahi D, Pratley RE, Tobin JD, Andres R. An epidemiological test of the hyperinsulinemia–hypertension hypothesis. *J Clin Endocrinol Metab* 1993; 76: 544–8.

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SEARCH REQUEST FORM

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Lipophilic microparticles containing a protein drug or antigen and formulation comprising same

Inventors (please provide full names): Myung-Jin KIM; Sun-Jin KIM; Kyu-chan KWON; Joon KIM

Earliest Priority Filing Date: ~~0~~ 1/18/99

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Please include an inventor name search.
Please search for a lipophilic microparticle that is 1-50μm that is a lipid, lipid derivative, fatty acid, Lipid is lecithin, phosphatidylcholine. Fatty acid is found in claim 7. The lipophilic particle also has hyaluronic acid that is selected from claim 9 and a water soluble excipient (claim 11). ~~Contains a drug~~ The particle contains a drug, such as growth hormone, or an antigen from any virus.

Thank you!

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Technical Info. Specialist
CM1 12D16 Tel: 308-4258

1539
1515-25, 31

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PTO-1590 (1-2000)

What is claimed is:

1. A lipophilic microparticle having an average particle size ranging from 0.1 to 200 μm, comprising a lipophilic substance and an active ingredient selected from the group consisting of a protein or peptide drug and an antigen.

2. The lipophilic microparticle of claim 1, wherein the average particle size is in the range of 1 to 50 μm.

3. The lipophilic microparticle of claim 1, wherein the drug is selected from the group consisting of human growth hormone, bovine growth hormone, porcine growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, macrophage-colony stimulating factor, erythropoietin, bone morphogenic protein, interferon, insulin, atriopeptin-III, monoclonal antibody, tumor necrosis factor, macrophage activating factor, interleukin, tumor degenerating factor, insulin-like growth factor, epidermal growth factor, tissue plasminogen activator and urokinase.

4. The lipophilic microparticle of claim 1, wherein the antigen is obtained from: one or more pathogens selected from the group consisting of adenovirus type 4&7, hepatitis A virus, hepatitis B virus, hepatitis C virus, influenza A & B virus, Japanese B encephalitis virus, measles virus, epidemic parotitis virus, rubella virus, polio virus, hydrophobia virus, chickenpox virus, yellow fever virus and human immunodeficiency virus; one or more pathogens selected from the group consisting of Bordetella pertussis, Borrelia burgdorferi, enterotoxigenic Escherichia coli, Haemophilus influenza type b, Mycobacterium leprae, Mycobacterium tuberculosis, Neisseria meningitidis A & C, Neisseria

meningitidis B, Pseudomonas aeruginosa, Pseudomonas cepacia, Salmonella typhi, Shigella spp., Streptococcus pneumoniae and Vibrio cholerae; one or more pathogens selected from the group consisting of Coccidiodes immitis, Leishmania sp. and Plasmodium sp.; or one or more pathogens responsible for the disease selected from the group consisting of bovine blackleg, bovine epidemic fever, bovine anthrax, bovine Akabane's disease, bovine foot-and-mouth disease, bovine mammitis, bovine infectious nasotracheal inflammation, bovine viral diarrhea, bovine infectious gastroenteritis, porcine cholera, porcine epidemic diarrhea, porcine atrophic gastritis, porcine disease caused by pavovirus, porcine enteritis caused by rotavirus, chicken Newcastle disease, chicken Marek's disease, chicken encephalomyelitis, rabies, dog distemper, dog enteritis caused by parvovirus and dog infectious hepatitis, the antigen being an attenuated, killed or recombinant antigen; or DNA, RNA, plasmid, CpG DNA or oligonucleotide extracted from the pathogen.

5. The lipophilic microparticle of claim 1, wherein the lipophilic substance is selected from the group consisting of a lipid, a lipid derivative, a fatty acid, a fatty acid derivative, a wax and a mixture thereof.

6. The lipophilic microparticle of claim 5, wherein the lipid is lecithin, phosphatidylcholine, phosphatidylehanolamine or phosphatidylserine, and the lipid derivative is arachidoyl phosphatidylcholine or stearoyl phosphatidylcholine.

7. The lipophilic microparticle of claim 5, wherein the fatty acid is myristic acid, palmitic acid or stearic acid, and the fatty acid derivative is glyceryl stearate, sorbitan palmitate, sorbitan stearate, sorbitan monooleate or polysorbate.

8. The lipophilic microparticle of claim 1, which further comprises hyaluronic acid or an inorganic salt thereof.

9. The lipophilic microparticle of claim 8, wherein the inorganic salt of hyaluronic acid is sodium hyaluronate, potassium hyaluronate, ammonium hyaluronate, calcium hyaluronate, magnesium hyaluronate, zinc hyaluronate or cobalt hyaluronate.

10. The lipophilic microparticle of any one of claims 1 and 8, which further comprises a water-soluble excipient.

11. The lipophilic microparticle of claim 10, wherein the water-soluble excipient is selected from group consisting of a carbohydrate, a protein, an amino acid, a fatty acid, an inorganic salt, a surfactant, poly(ethylene glycol) and a mixture thereof.

12. A dispersion formulation prepared by dispersing the lipophilic microparticle of any one of claims 1, 8 and 10 in a lipophilic medium.

13. The dispersion formulation of claim 12, wherein the lipophilic medium is an edible oil, mineral oil, squalene, squalane, cod liver oil, mono-, di- or triglyceride, or a mixture thereof.

14. The dispersion formulation of claim 13, wherein the edible oil is corn oil, olive oil, soybean oil, safflower oil, cotton seed oil, peanut oil, sesame oil, sunflower oil or a mixture thereof.

15. The dispersion formulation of claim 12, wherein the lipophilic medium further comprises a dispersing agent or a preservative.

16. The dispersion formulation of any one of claims 12 and 15, which is used for injection or oral administration.

17. An oil-in-water emulsion formulation comprising an aqueous injection medium and the dispersant formulation of claim 12.

18. The oil-in-water emulsion formulation of claim 17, wherein the aqueous injection medium is distilled water or a buffered solution.

19. The oil-in-water emulsion formulation of claim 17, wherein the active ingredient is an antigen and the aqueous injection medium further comprises a second antigen.

20. An aerosol formulation comprising the lipophilic microparticle of any one of claims 1 to 11.

Abstract

A lipophilic microparticle having an average particle size ranging from 0.1 to 200 μm, comprising a lipophilic substance and an active ingredient selected from the group consisting of a protein or peptide drug and an antigen, retains the full activity of the active ingredient, and when formulated in the form of an oil dispersion or oil-in-water emulsion, it releases in an in vivo environment the active ingredient in a controlled manner over a long period.

Foley
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=> s lipophilic microparticle? or lipid or fatty acid or wax or lecithin or phosphatidylcholine or phosphatidylethanolamine or phosphatidylserine or arachidoyl phosphatidylcholine or stearoyl phosphatidylcholine

L1 272569 FILE MEDLINE
L2 463541 FILE CAPLUS
L3 329800 FILE BIOSIS
L4 217199 FILE EMBASE
L5 103174 FILE WPIDS
L6 80788 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L7 1467071 LIPOPHILIC MICROPARTICLE? OR LIPID OR FATTY ACID OR WAX OR LECIT
HIN OR PHOSPHATIDYLCHOLINE OR PHOSPHATIDYLETHANOLAMINE OR PHOSPH
ATIDYLSERINE OR ARACHIDOYL PHOSPHATIDYLCHOLINE OR STEAROYL PHOSP
HATIDYLCHOLINE

=> s myristic acid or palmitic acid or stearic acid or glyceryl stearate or sorbitan palmitate or sorbitan stearate or sorbitan monooleate or polysorbate or hyaluronic acid or sodium hyaluronate or potassiu, hyaluronate or ammonium hyaluronate or calcium hyaluronate or magnesium hyaluronate

L8 23166 FILE MEDLINE
L9 62705 FILE CAPLUS
L10 17512 FILE BIOSIS
L11 21513 FILE EMBASE
L12 14540 FILE WPIDS
L13 3148 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L14 142584 MYRISTIC ACID OR PALMITIC ACID OR STEARIC ACID OR GLYCERYL STEAR
ATE OR SORBITAN PALMITATE OR SORBITAN STEARATE OR SORBITAN MONOO
LEATE OR POLYSORBATE OR HYALURONIC ACID OR SODIUM HYALURONATE OR POTASSIU, HYALURONATE OR AMMONIUM HYALURONATE OR CALCIUM HYALURONATE OR MAGNESIUM HYALURONATE

=> s zinc hyaluronate or cobalt hyaluronate

L15 1 FILE MEDLINE
L16 9 FILE CAPLUS
L17 1 FILE BIOSIS
L18 1 FILE EMBASE
L19 4 FILE WPIDS
L20 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L21 16 ZINC HYALURONATE OR COBALT HYALURONATE

=> s (l7 or l14 or l21) and ((human or bovine or porcine)(w)hormone or growth hormone releas?(w)(hormone or peptide) or granulocyte(w)(colony stimulat? or macrophage colony)(w)stimulat? factor or interferon or insulin or monoclonal antibod?)

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L22       22885 FILE MEDLINE
L23       18604 FILE CAPLUS
L24       19000 FILE BIOSIS
L25       20589 FILE EMBASE
L26        1139 FILE WPIDS
L27        4322 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L28       86539 (L7 OR L14 OR L21) AND ((HUMAN OR BOVINE OR PORCINE)(W)
HORMONE
                OR GROWTH HORMONE RELEAS?(W)(HORMONE OR PEPTIDE) OR
GRANULOCYTE(
                W)(COLONY STIMULAT? OR MACROPHAGE COLONY)(W) STIMULAT? FACTOR
                OR INTERFERON OR INSULIN OR MONOCLONAL ANTIBOD?)

=> s (l7 or l14 or l21) and (tumor necrosis or macrophage activat? or tumor
degenerat? or epidermal growth factor or tissue plasminogen activator or
urokinase)

L29        3147 FILE MEDLINE
L30        3066 FILE CAPLUS
L31        2805 FILE BIOSIS
L32        3600 FILE EMBASE
L33         238 FILE WPIDS
L34         488 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L35       13344 (L7 OR L14 OR L21) AND (TUMOR NECROSIS OR MACROPHAGE ACTIVAT?
                OR TUMOR DEGENERAT? OR EPIDERMAL GROWTH FACTOR OR TISSUE
PLASMIN
                OGEN ACTIVATOR OR UROKINASE)

=> s (l28 or l35) and (antigen or adenovirus tyoe(w)(4 or 7) or hepatitis or
influenza or japanese b encephalitis or (measle or epidemic parotitis or
rubella or polio or hydrophobia or chickenpox or yellow fever or human
immunodeficienc?)(w)virus)

L36        1902 FILE MEDLINE
L37        1613 FILE CAPLUS
L38        1110 FILE BIOSIS
L39        2078 FILE EMBASE
L40         251 FILE WPIDS
L41         644 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L42        7598 (L28 OR L35) AND (ANTIGEN OR ADENOVIRUS TYOE(W)(4 OR 7) OR
HEPAT
                ITIS OR INFLUENZA OR JAPANESE B ENCEPHALITIS OR (MEASLE OR
EPIDE
                MIC PAROTITIS OR RUBELLA OR POLIO OR HYDROPHOBIA OR CHICKENPOX
                OR YELLOW FEVER OR HUMAN IMMUNODEFICIENC?)(W) VIRUS)

=> s (l28 or l35) and (bordetell? pertuss? or borrel? burgdorf? or
escherichia coli or haemophilus influenza or mycobacter? lepra? or
mycobacter? tuberculos? or neisser? meningitid? or pseudomonas aeruginos? or
pseudomonas cepaci? or salmonella typhi or shigella? or streptococc? pneumon?)
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L43         526 FILE MEDLINE
L44         485 FILE CAPLUS
L45         447 FILE BIOSIS
L46         436 FILE EMBASE
L47          31 FILE WPIDS
L48          91 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L49        2016 (L28 OR L35) AND (BORDETELL? PERTUSS? OR BORREL? BURGDORF? OR
                ESCHERICHIA COLI OR HAEMOPHILUS INFLUENZA OR MYCOBACTER?
LEPRA?
                OR MYCOBACTER? TUBERCULOS? OR NEISSER? MENINGITID? OR
PSEUDOMONA
                S AERUGINOS? OR PSEUDOMONAS CEPACI? OR SALMONELLA TYPHI OR
SHIGE
                LLA? OR STREPTOCOCC? PNEUMON?)

=> s (l28 or l35) and (vibrio choler? or coccidiode? immit? or leishman? or
plasmodium or bovine(w)(blackleg or epidemic fever or anthrax or akabane? or
foot mouth or mammitis or infectious nasotracheal inflam? or viral
diarrhea)(w)disease)

L50          64 FILE MEDLINE
L51          88 FILE CAPLUS
L52          67 FILE BIOSIS
L53          89 FILE EMBASE
L54          20 FILE WPIDS
L55           5 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L56         333 (L28 OR L35) AND (VIBRIO CHOLER? OR COCCIDIODE? IMMIT? OR
LEISHM
                AN? OR PLASMODIUM OR BOVINE(W)(BLACKLEG OR EPIDEMIC FEVER OR
                ANTHRAX OR AKABANE? OR FOOT MOUTH OR MAMMITIS OR INFECTIOUS
                NASOTRACHEAL INFLAM? OR VIRAL DIARRHEA)(W) DISEASE)

=> s (l28 or l35) and (porcine(w)(cholera or epidemic diarrhea or atrophic
gastritis or disease) or pavovirus or chicken newcastle or chicken marek? or
chicken encephalomyelit? or rabies or dog(w)(enteritis or distemper)) or

MISSING TERM AFTER ) OR
Operators must be followed by a search term, L-number, or query name.

=> s (l28 or l35) and (porcine(w)(cholera or epidemic diarrhea or atrophic
gastritis or disease) or pavovirus or chicken newcastle or chicken marek? or
chicken encephalomyelit? or rabies or dog(w)(enteritis or distemper))

L57           4 FILE MEDLINE
L58          13 FILE CAPLUS
L59           7 FILE BIOSIS
L60           5 FILE EMBASE
L61           8 FILE WPIDS
L62           1 FILE JICST-EPLUS

TOTAL FOR ALL FILES
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```
L63          38 (L28 OR L35) AND (PORCINE(W)(CHOLERA OR EPIDEMIC DIARRHEA OR
                ATROPHIC GASTRITIS OR DISEASE) OR PAVOVIRUS OR CHICKEN
NEWCASTLE
                 OR CHICKEN MAREK? OR CHICKEN ENCEPHALOMYELIT? OR RABIES OR
                DOG(W)(ENTERITIS OR DISTEMPER))

=> s (l63 or l56 or l49 or l42) and (water soluble or carbohydrate or protein
or amino acid or fatty acid or inorganic salt or surfactant or polyethylene
glycol)

L64        1400 FILE MEDLINE
L65        1215 FILE CAPLUS
L66         716 FILE BIOSIS
L67        1319 FILE EMBASE
L68         198 FILE WPIDS
L69         423 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L70        5271 (L63 OR L56 OR L49 OR L42) AND (WATER SOLUBLE OR CARBOHYDRATE
                OR PROTEIN OR AMINO ACID OR FATTY ACID OR INORGANIC SALT OR
                SURFACTANT OR POLYETHYLENE GLYCOL)

=> s l70 and ((edible or mineral or cod liver or corn or olive or soybean or
safflower or cotton seed or peanut or sesame or sunflower)(w)oil or squalene
or squalane or ?triglyceride?)

L71         115 FILE MEDLINE
L72          70 FILE CAPLUS
L73          59 FILE BIOSIS
L74         115 FILE EMBASE
L75          11 FILE WPIDS
LEFT TRUNCATION IGNORED FOR '?TRIGLYCERIDE?' FOR FILE 'JICST-EPLUS'
L76          16 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L77         386 L70 AND ((EDIBLE OR MINERAL OR COD LIVER OR CORN OR OLIVE OR
                SOYBEAN OR SAFFLOWER OR COTTON SEED OR PEANUT OR SESAME OR
SUNFL
                OWER)(W) OIL OR SQUALENE OR SQUALANE OR ?TRIGLYCERIDE?)
Left truncation is not valid in the specified search field in the
specified file.  The term has been searched without left truncation.
Examples: '?TERPEN?' would be searched as 'TERPEN?' and '?FLAVONOID'
would be searched as 'FLAVONOID.'

If you are searching in a field that uses implied proximity, and you
used a truncation symbol after a punctuation mark, the system may
interpret the truncation symbol as being at the beginning of a term.
Implied proximity is used in search fields indexed as single words,
for example, the Basic Index.

=> s l77 and (oil dispers? or oil(2w)water or inject? or oral? or dispers?)

L78          15 FILE MEDLINE
L79          20 FILE CAPLUS
L80           9 FILE BIOSIS
L81          27 FILE EMBASE
```

L82 8 FILE WPIDS
L83 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L84 79 L77 AND (OIL DISPERS? OR OIL(2W) WATER OR INJECT? OR ORAL? OR DISPERS?)

=> dup rem l84

PROCESSING COMPLETED FOR L84
L85 53 DUP REM L84 (26 DUPLICATES REMOVED)

=> d 1-53 cbib abs;s kim m?/au,in;s kim s?/au,in;s kwon k?/au,in;s kim j?/au,in

L85 ANSWER 1 OF 53 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
2000:493356 Document No. 133:125288 **Lipophilic microparticles** containing a **protein** drug or **antigen** and formulation comprising same for controlled release. Kim, Myung Jin; Kim, Sun Jin; Kwon, Kyu Chan; Kim, Joon (LG Chemical Limited, S. Korea). PCT Int. Appl. WO 2000041682 A1 20000720, 49 pp. DESIGNATED STATES: W: AU, BG, BR, CA, CN, HU, ID, IL, IN, JP, MX, NZ, PL, SG, TR, US, ZA; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-KR25 20000114. PRIORITY: KR 1999-1232 19990118; KR 1999-59776 19991221.
AB A **lipophilic microparticle** having an av. particle size ranging from 0.1 to 200 .mu.m, comprising a lipophilic substance and an active ingredient selected from the group consisting of a **protein** or peptide drug and an **antigen**, retains the full activity of the active ingredient, and when formulated in the form of an **oil dispersion** or **oil**-in-**water** emulsion, it releases in an in vivo environment the active ingredient in a controlled manner over a long period.

L85 ANSWER 2 OF 53 CAPLUS COPYRIGHT 2001 ACS
2000:259972 Document No. 132:293042 Encapsulation of sensitive liquid components into a matrix to obtain discrete shelf-stable particles. Van Lengerich, Bernhard H. (General Mills, Inc., USA). PCT Int. Appl. WO 2000021504 A1 20000420, 56 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20905 19991006. PRIORITY: US 1998-PV103700 19981009; US 1998-PV109696 19981124; US 1999-233443 19990120.
AB A liq. encapsulant component which contains an active, sensitive encapsulant, such as a live microorganism or an enzyme dissolved or **dispersed** in a liq. plasticizer is admixed with a plasticizable

matrix material. The matrix material is plasticizable by the liq. plasticizer and the encapsulation of the active encapsulant is accomplished at a low temp. and under low shear conditions. The active component is encapsulated and/or embedded in the plasticizable matrix component or material in a continuous process to produce discrete, solid particles. The liq. content of the liq. encapsulant component provides substantially all or completely all of the liq. plasticizer needed to plasticize the matrix component to obtain a formable, extrudable, cuttable, mixt. or dough. Removal of liq. plasticizer prior to extrusion is not needed to adjust the viscosity of the mixt. for formability. Release of an active component from the matrix may be delayed or controlled over time so that the active component is delivered when and where it is needed to perform its intended function. Controlled release, discrete, solid particles which contain an encapsulated and/or embedded component such as a heat sensitive or readily oxidizable pharmaceutically, biol., or nutritionally active component are continuously produced without substantial destruction of the matrix material or encapsulant.

L85 ANSWER 3 OF 53 CAPLUS COPYRIGHT 2001 ACS
2000:190953 Document No. 132:241914 Immunostimulant emulsions comprising polynucleotides. Haensler, Jean (Pasteur Merieux Serums Et Vaccins, Fr.).

PCT Int. Appl. WO 2000015256 A2 20000323, 18 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (French). CODEN: PIXXD2. APPLICATION: WO 1999-FR2177 19990913. PRIORITY: FR 1998-11520 19980911.

AB The invention concerns an **oil**-in-**water** immunostimulant emulsion comprising an aq. phase and an oil phase, characterized in that it further comprises an immunostimulant polynucleotide whereof at least part is covalently bound to at least a **lipid** mol. The invention also concerns a vaccine compn. comprising such an emulsion as immunoadjuvant. An immunostimulant emulsion was obtained by mixing 435 .mu.L of a soln. of 2.3 g/L of oligonucleotide 3Db(S) (all phosphorothiolate linkages) conjugated with cholesterol with 2 mL of an emulsion of **squalene**/PBS. The immunostimulant efficacy of the emulsion was shown in guinea pigs.

L85 ANSWER 4 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-505719 [45] WPIDS
AB WO 200041740 A UPAB: 20000918
NOVELTY - Particle (A) for **oral** delivery of a biopolymeric drug (I) (e.g. polypeptide, **protein** or nucleic acid), comprising a substrate having at least 1 reservoir containing (I) in releasable form and opening to 1 face of the substrate, which is coated with a mucoadhesive agent (II) for the attachment of (A) to the intestinal mucosa so that (I) is released directly into the lining, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an **oral** composition containing many (A); and

(2) a microfabrication method comprising exposing a sheet of particle-forming material to a photoablative light source through a mask, so that a network pattern corresponding to the required shape and size of (A) is produced, and continuing exposure until (A) are formed.

USE - (A) are used for the **oral** delivery of (I) to the intestines, e.g., the delivery of erythropoietin (for treating anemia), **interferons** (**hepatitis**), interleukins (cancer), **insulin** (diabetes mellitus), calcitonin (osteoporosis) and antisense oligonucleotides (cancer, infections, inflammation).

ADVANTAGE - (II) ensure attachment to the intestines and their shape, size, density and composition can be adjusted to control contact with the gut wall. (A) are too large to undergo endocytosis by gut epithelial cells and they can be labeled for detection or visualization. They may also include penetration enhancers; protease inhibitors or agents that control release rate of (I), to improve bioavailability.

Dwg.0/8

L85 ANSWER 5 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 2000-349917 [30] WPIDS

AB WO 200020027 A UPAB: 20000624

NOVELTY - A method (I) for inducing immune responses against weakly immunogenic cell-associated peptide **antigens** (PA) such as those associated with cancers (i.e. self-**proteins**) (e.g. human PSM (undefined), Her2 and/or fibroblast growth factor (FGF) 8b), is new.

DETAILED DESCRIPTION - A method (I) for inducing an immune responses against weakly immunogenic or non-immunogenic polypeptide **antigens** (PAs) in animals (including humans), comprising effecting simultaneous presentation by **antigen** producing cells (APCs) of the animals immune system of:

(1) at least 1 CTL (cytotoxic T-lymphocyte) group derived from the PA and/or at least 1 B-cell group derived from the cell-associated PA; and

(2) at least 1 first T helper cell group (TH1 group) which is foreign to the animal.

INDEPENDENT CLAIMS are also included for the following:

(1) a method (II) for the selection of an immunogenic analog of a cell-associated PA that is weakly immunogenic or non-immunogenic which is capable of inducing an immune response in an animal against cell displaying MHC (major histocompatability complex) Class I (MHC-I) molecules bound to group derived from the cell-associated PA, comprising:

(A) identifying a subsequence of the **amino acid** sequence of the cell-associated PA which does not contain known or predicted CTL groups;

(B) preparing at least 1 punitively immunogenic analogs of the PA by introducing at least 1 TH group foreign to the animal in a position within the subsequence identified in step (A); and

(C) selecting those analogs from step (B) which are verifiably capable of inducing a CTL response in the animal

(2) a method (III) for the preparation of a cell that produces analogs of cell-associated PAs, comprising introducing a nucleic acid encoding the analog into a vector and transforming a suitable host cell (III) with the vector;

(3) a method (IV) for preparing analogs of cell-associated PAs comprising culturing the transformed host cell (III) under conditions suitable for expression of the **protein** and recovering the PA analog from the culture;

(4) an analog (V) of human PSM (undefined) that is immunogenic in humans and comprises at least part of all known and predicted CTL and B-cell groups of PSM and includes at least 1 foreign TH group;

(5) an analog (VI) of Her2 that is immunogenic in humans and comprises at least part of all known and predicted CTL and B-cell groups of Her2 and includes at least 1 foreign TH group;

(6) an analog (VII) of human/murine FGF (fibroblast growth factor) 8b that is immunogenic in humans and comprises at least part of all known and predicted CTL and B-cell groups of FGF 8b and includes at least 1 foreign TH group;

(7) compositions comprising (V), (VI) and/or (VII) and an adjuvant;

(8) nucleic acids ((VIII)-(X)) encoding (V), (VI) and/or (VII);

(9) vectors ((XI)-(XIII)) comprising (VIII)-(X) (respectively);

(10) a transformed cell (XIV) comprising (XI)-(XIII);

(11) compositions for inducing production of antibodies against PSM, Her2 and FGF 8b, comprising (VIII)-(X) and/or (XI)-(XIII) and an adjuvant;

and

(12) a method for the preparation of the cell (XIV), comprising transforming a host cell with (VIII)-(X) or (XI)-(XIII).

USE - (I) is used to stimulate immune responses to weakly, or non-immunogenic peptide **antigens** especially self **proteins** for the treatment of diseases associated with expression of those **antigens**. If the PA is human PSM (undefined), (I) is used for the treatment of prostate cancer. If the PA is human fibroblast growth factor (FGF) 8b, (I) is used for the treatment of prostate cancer or breast cancer. If the PA is Her2, (I) is used for the treatment of breast cancer (claimed).

Dwg.0/6

L85 ANSWER 6 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 2000-430219 [37] WPIDS

AB US 6071534 A UPAB: 20000807

NOVELTY - Multivesicular liposome having non-concentric chambers with membranes distributed in a matrix is produced by **dispersing** a water in oil emulsion comprising a **lipid** component, aqueous component and a hydrochloride into a second aqueous component.

DETAILED DESCRIPTION - Multivesicular liposome having non-concentric chambers with membranes distributed in a matrix is produced by:

(1) forming a water-in-oil emulsion from a **lipid** component comprising an organic solvent, an amphipathic **lipid** and a neutral **lipid** lacking a hydrophilic head group and an aqueous component and which contains 10-500 mM hydrochloric acid, arginine hydrochloride, histidine hydrochloride, lysine hydrochloride and/or pyridine hydrochloride, and at least one biologically active substance;

(2) **dispersing** the water-in-oil emulsion into a second aqueous component to form solvent spherules and

(3) removing the organic solvent from the spherules to form the liposomes suspended in the second aqueous component.

The concentration of hydrohalide is selected to modulate the in vivo

release rate of the biologically active substance.

USE - Useful for the controlled release of active agents encapsulated in the presence of a hydrochloride. The liposome is used to give prolonged and sustained in vivo exposure at a disease site of a therapeutic concentration of the active substance

ADVANTAGE - The liposomes provide high encapsulation efficiency, controlled release rate, well defined, reproducible size distribution and adjustable internal chamber size and number.

Dwg.0/1

L85 ANSWER 7 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-136656 [12] WPIDS
CR 1998-387767 [33]
AB US 6017513 A UPAB: 20000308

NOVELTY - Mucosal administration of substances to mammals comprises contacting a mucosal surface with the substance in combination with Biovector core comprising natural polymer and/or its hydrolysate that is uncoated or partially or completely coated with no more than one layer comprising **lipid** compound covalently bonded to the core or an amphiphilic compound.

ACTIVITY - Drug delivery.

The delivery system is used for mucosal administration of substances to mammals (claimed) including therapeutic or prophylactic agents (radiopharmaceutical, analgesic, anesthetic, anorectic, anti-anemic, anti-asthmatic, anti-diabetic, antihistamine, anti-inflammatory, antibiotics, antimuscarinic, antineoplastic, antiviral, cardiovascular drug, CNS depressant, CNS stimulator, antidepressant, anti-epileptic, anxiolytic, hypnotic, sedative, anti-psychotic, beta blocker, hemostatic, hormone, vasodilator, vasoconstrictor, vitamin, vaccines against pathogens including bacteria, viruses, yeasts or fungi, specifically **influenza** virus (preferred), cytomegalovirus, **human immunodeficiency virus**, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, **measles virus**, arbor virus, Coxsackie virus, herpes virus, hantavirus, **hepatitis** virus, Lyme disease virus, mumps virus or rotavirus, Neisseria, Aerobacter, Pseudomonas, Porphyromonas, Salmonella, Escherichia, Pasteurella, **Shigella**, Bacillus, Helibacter (sic), Corynebacterium, Clostridium, Mycobacterium, Yersinia, Staphylococcus, Bordetella, Brucella, Vibrio or Streptococcus, **Plasmodium**, Schisostoma or Candida, diagnostic agents such as contrast or imaging agent e.g. that detect corneal irregularities or those labeled with detectable groups (radioactive, magnetic or fluorescent), small chemical molecules (organic, inorganic or organo-metallic molecules) or biological molecules (**amino acid**, oligopeptide, peptide, **protein**, glycoprotein, lipoprotein, proteoglycan, lipopolysaccharide, **fatty acid**, eicosanoid, **lipid**, **triglyceride**, phospholipid, glycolipid, nucleoside, nucleotide, nucleic acid, DNA molecule, RNA molecule, monosaccharide, oligosaccharide or polysaccharide) (claimed) as well as cytokines, growth factors, enzymes, **antigens** (including epitopes of **antigens** and haptens), antibodies, hormones, (natural and synthetic hormones and their derivatives), co-factors, receptors, enkephalins, endorphins, neurotransmitter s and nutrients such as

insulin, **interferon** (alpha -, beta - or gamma -IFN), interleukins (IL-1 to IL-15), interleukin receptors (IL-1 receptor), calcitonin, erythropoeitin, thrombopoietin, **epidermal growth factor** and **insulin**-like growth factor-1. It can also be used to detect irregularities within the respiratory tract, digestive tract, auditory canal, urethra, rectum or any part of mammal with mucosal membrane and to deliver contrast agents for magnetic resonance imaging.

Influenza hemagglutinin (HA) was delivered by Biovectors to female mice, with 5 mu g HA applied intranasally in 20 or 50 mu l of phosphate-buffered saline solution or suspension, either alone or in Biovector formulation. One group of animals was subjected to light ether anesthesia, while the others were awake. Administration of 20 mu l on the outer nostrils of awake animals restricted the **antigen** to the upper respiratory tract. Volume of 50 mu l directly into the nostrils of anesthetized animals resulted in deposition of at least some **antigen** in the lower respiratory tract and lung as well as in the nasal cavity. Four different Biovectors were used: (Q) positive and (P) negatively charged light Biovectors either re-suspended - (1Q) and (1P) - or **dispersed** (2Q) and (2P). **Influenza** virus subunit **antigen** was either pre-loaded in the Biovectors or post-loaded (admixed immediately prior to administration). **Antigen** alone was used as control (3). Mice were sacrificed at day 28 and serum samples from the vena porta analyzed by direct enzyme-linked immunosorbent assay (ELISA). Serum immunoglobulin G titers (geometric mean) determined as reciprocal of sample dilution corresponding to absorbance at 492 nm of 0.2 above background were as follows in unanesthetized animals: (1Q) pre-loaded = 10, post-loaded = 20; (1P) pre-loaded = 400, post-loaded = 30; (2Q) pre- and post-loaded = 2,000; (2P) pre- and post-loaded = 200, and as follows in anesthetized animals: (1Q) = 30; (1P) = 30; (2Q) 4,000; (2P) = 60. Control was 100 for unanesthetized animals and 200 in anesthetized animals.

MECHANISM OF ACTION - None given.

ADVANTAGE - The system is capable of delivering substances to animals (including humans) efficiently and avoiding disadvantages of the prior art. The carrier directs substances to the mucosa in a non-specific manner, is capable of being loaded with the substance immediately prior to administration, is of a size susceptible to microfiltration for sterilization avoiding need for preservatives and it is stable for up to 12 months, even one or more years. Biovectors have larger relative surfaces and volumes than larger microspheres or nanospheres and multiple substances can be delivered per Biovector.

Dwg.0/11

L85 ANSWER 8 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

2000178196 EMBASE The conformation of apolipoprotein A-I in high-density lipoproteins is influenced by core **lipid** composition and particle size: A surface plasmon resonance study. Curtiss L.K.; Bonnet D.J.; Rye K.-A.. L.K. Curtiss, Department of Immunology, Scripps Research Institute, 10550 North Torrey Pines Road, San Diego, CA 92037, United States. lcurtiss@scripps.edu. Biochemistry 39/19 (5712-5721) 16 May

2000.
Refs: 33.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.

AB Plasma high-density lipoproteins (HDL) are a heterogeneous group of particles that vary in size as well as **lipid** and apoprotein composition. The effect of HDL core **lipid** composition and particle size on apolipoprotein (apo) A-I structure was studied using surface plasmon resonance (SPR) analysis of the binding of epitope-defined **monoclonal antibodies**. The association and dissociation rate constants of 12 unique apo A-I-specific **monoclonal antibodies** for isolated plasma HDL were calculated. In addition, the association rate constants of the antibodies were determined for homogeneous preparations of spherical reconstituted HDL (rHDL) that contained apo A-I as the sole apolipoprotein and differed either in their size or in their core **lipid** composition. This analysis showed that lipoprotein size affected the conformation of domains **dispersed** throughout the apo A-I molecule, but the conformation of the central domain between residues 121 and 165 was most consistently modified. In contrast, replacement of core cholesteryl esters with **triglyceride** in small HDL modified almost the entire molecule, with only two key N-terminal domains of apo A-I being unaffected. This finding suggested that the central and C-terminal domains of apo A-I are in direct contact with rHDL core **lipids**. This immunochemical analysis has provided valuable insight into how core **lipid** composition and particle size affect the structure of specific domains of apo A-I on HDL.

L85 ANSWER 9 OF 53 MEDLINE

2000473363 Document Number: 20416287. Dietary fat alters HIV protease inhibitor-induced metabolic changes in mice. Lenhard J M; Croom D K; Weiel J E; Spaltenstein A; Reynolds D J; Furfine E S. (Department of Metabolic Diseases, Glaxo Wellcome Incorporated, Research Triangle Park, NC 27709, USA.) JOURNAL OF NUTRITION, (2000 Sep) 130 (9) 2361-6. Journal code: JEV. ISSN: 0022-3166. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** (HIV) protease inhibitors (PI) may alter **lipid** metabolism in patients with acquired immunodeficiency syndrome (AIDS). However, the influence of dietary fat on the metabolic effects of PI therapy remains unknown. AKR/J mice were fed high or low fat diets and treated with the PI indinavir (IDV), nelfinavir (NFV), saquinavir (SQV) or amprenavir (APV) by subcutaneous delivery for 2 wk. Serum concentrations of glucose, **insulin, triglyceride**, free **fatty acid** , glycerol, pancreatic lipase, bilirubin, alkaline phosphatase, blood urea nitrogen and PI, and interscapular and epididymal fat weights were determined. Some metabolic effects of PI were dependent on diet. IDV- and NFV-treated mice had greater serum glucose concentration and body weight; IDV-treated mice had lower serum **insulin**; NFV-treated mice had greater interscapular fat mass; and SQV treated mice had lower serum **triglyceride** concentration than control mice fed the low but not the high fat diet. In contrast, NFV- and IDV-treated mice had greater **triglyceride** concentration and blood urea nitrogen, and SQV treated mice had greater serum cholesterol than control mice fed the high

but not the low fat diet. The serum concentration of SQV was lower in mice fed the high fat compared with the low fat diet. Other effects were not dependent on diet. IDV- and NFV-treated mice had greater **fatty acids**, and IDV-treated mice had greater pancreatic lipase, bilirubin and alkaline phosphatase than control mice fed either diet. APV treatment had little effect on these serum measurements. Thus, changes in dietary fat can influence some but not all of the effects of PI on metabolism. Furthermore, each PI produces different effects in vivo, indicating that various PI affect distinct metabolic pathways.

L85 ANSWER 10 OF 53 MEDLINE DUPLICATE 2
2000322430 Document Number: 20322430. Effect of hyperglycemia and hyperlipidemia on atherosclerosis in LDL receptor-deficient mice: establishment of a combined model and association with heat shock **protein** 65 immunity. Keren P; George J; Shaish A; Levkovitz H; Janakovic Z; Afek A; Goldberg I; Kopolovic J; Keren G; Harats D. (Institute of Lipid and Atherosclerosis Research, Sheba Medical Center, Tel-Hashomer, Israel.) DIABETES, (2000 Jun) 49 (6) 1064-9. Journal code: E8X. ISSN: 0012-1797. Pub. country: United States. Language: English.
AB Diabetes and atherosclerosis have been proposed to be influenced by immune and autoimmune mechanisms. A common incriminated **antigen** in both disorders is the heat shock **protein** (HSP)-60/65. In the current study, we established a model combining hyperglycemia with hyperlipidemia in LDL receptor-deficient (LDL-RD) mice and assessed its possible influences on **lipid** profile, HSP60/65, and atherogenesis. LDL-RD mice were **injected** either with streptozotocin to induce hyperglycemia or with citrate buffer (control). When hyperglycemia was induced, both study groups were challenged with a high-fat (Western) diet for 6 weeks. Plasma fasting glucose, **lipid** profile, and antibody levels to HSP65 and oxidized LDL were assessed. At death, the spleens from both groups were evaluated for their proliferative response to HSP65 and the consequent cytokine production. The extent of atherosclerosis was assessed at the aortic sinus. Plasma glucose, cholesterol, and **triglyceride** levels were elevated in mice **injected** with streptozotocin compared with control mice. Atherosclerotic lesions were significantly larger in the streptozotocin-**injected** hyperglycemic LDL-RD mice (132 +/- 23 x 10(5) microm2) in comparison to their normoglycemic litter-mates (20 +/- 6.6 x 10(5) microm2; P < 0.0001). Both humoral and cellular immune response to HSP65 was more pronounced in streptozotocin-**injected** mice. When challenged with HSP65 in vitro, splenocytes from streptozotocin-**injected** mice favored the production of the T-helper (TH)-1 cytokine gamma-**interferon**. In conclusion, we have established a mouse model that combines hyperglycemia with diet-induced hyperlipidemia in LDL-RD mice and studied its effect on atherosclerosis progression. The accelerated atherosclerotic process is associated with heightened immune response to HSP65 and a shift to a TH1 cytokine profile.

L85 ANSWER 11 OF 53 MEDLINE DUPLICATE 3
2000177936 Document Number: 20177936. Inhibition of CD11-CD18 complex prevents acute lung injury and reduces mortality after peritonitis in

rabbits. Gardinali M; Borrelli E; Chiara O; Lundberg C; Padalino P; Conciato L; Cafaro C; Lazzi S; Luzi P; Giomarelli P P; Agostoni A. (Dipartimento di Medicina Interna and Istituto di Chirurgia d'Urgenza, IRCCS Ospedale Policlinico, Universit`a di Milano, Milano, Italy.) AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, (2000 Mar) 161 (3 Pt 1) 1022-9. Journal code: BZS. ISSN: 1073-449X. Pub. country: United States. Language: English.

AB Acute lung injury is frequent after severe peritonitis. The aim of this study was to investigate whether inhibition of the adhesion molecule CD11-CD18 on polymorphonuclear leukocytes (PMNs) would have any beneficial effects on pulmonary function and mortality in an animal model reproducing these clinical conditions. Acute peritonitis was induced in 36 rabbits by intraperitoneal **injection** of zymosan (0.6 g/kg) suspended in **mineral oil**; 20 were pretreated with a murine-specific IgG2a anti-CD18 **monoclonal antibody**, 16 (controls) with nonspecific purified murine IgG (1 mg/kg). The animals were followed for 10 d, then killed for histologic examination of the lungs. Blood samples were taken on Days 0, 1, 3, 7, and 10 for red blood cell (RBC), white blood cell (WBC), and platelet counts, pH, PO(2), PCO(2), carbon dioxide content (HCO(3)(-)) measurements, and renal and liver tests. Treatment with the anti-CD18 **monoclonal antibody** reduced mortality by approximately 40% ($p < 0.05$). PO(2) was higher in these treated animals than in the control animals throughout the study ($p < 0.05$ on Day 1, 3, and 10). On Day 1 control animals had significant leukopenia, whereas anti-CD18-treated animals had a moderate increase of the number of circulating WBC compared with baseline values ($p < 0.05$ between groups). The lungs of the anti-CD18-treated animals showed minor signs of inflammation and PMN infiltration whereas controls had interstitial and intra-alveolar edema and a large number of granulocytes. Quantification of PMNs by morphometry showed that there were constantly less granulocytes in the lungs of the animals treated with the anti-CD18 antibody ($p < 0.001$). PMN infiltration correlated with the levels of PO(2) ($p < 0.001$). Lung tissue of anti-CD18-treated rabbits contained less malonyldialdehyde, a by-product of membrane **lipid** peroxidation by PMN oxygen radicals (950 +/- 120 versus 1,710 +/- 450 pM/mg of **protein**) and, conversely, more of the antioxidant alpha-tocopherol (136 +/- 22 versus 40 +/- 9 ng/mg of **protein**), than the control rabbits ($p < 0.01$). In this particular model of ARDS the **monoclonal antibody** against the CD11-CD18 complex had a beneficial effect, reducing PMN infiltration and oxygen radical release in the lungs, preventing alveolocapillary membrane damage, improving gas exchange and, finally, significantly reducing mortality.

L85 ANSWER 12 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

2000043494 EMBASE Effects of gemfibrozil on **insulin** sensitivity and on haemostatic variables in **hypertriglyceridemic** patients.

Mussoni L.; Mannucci L.; Sirtori C.; Pazzucconi F.; Bonfardeci G.; Cimminiello C.; Notarbartolo A.; Scafidi V.; Bittolo Bon G.; Alessandrini P.; Nenci G.; Parise P.; Colombo L.; Piliego T.; Tremoli E.. E. Tremoli, Institute Pharmacological Sciences, E. Grossi Paoletti Center, University

of Milan, Via Balzaretti 9, 20133 Milan, Italy. elena.tremoli@unimi.it.
Atherosclerosis 148/2 (397-406) 2000.
Refs: 64.
ISSN: 0021-9150. CODEN: ATHSBL.
Publisher Ident.: S 0021-9150(99)00283-X. Pub. Country: Ireland.
Language:
English. Summary Language: English.
AB In order to assess the efficacy of gemfibrozil on **lipid** and haemostatic parameters in patients with plurimetabolic syndrome, a multicenter double-blind placebo controlled, parallel study was carried out in 56 patients with primary **hypertriglyceridemia** and glucose intolerance. These patients had elevated PAI activity and **antigen** and t-PA **antigen** levels at rest and after venous occlusion. Gemfibrozil reduced plasma **triglyceride** levels (P<0.001), whereas it increased free **fatty acids** (P<0.05) and high density lipoprotein cholesterol levels (P<0.05). In those patients reaching normalization of plasma **triglyceride** levels (**triglyceride** reduction .gtoreq. 50%) (n=15), **insulin** levels (P<0.05) as well as the **insulin** resistance index were reduced by gemfibrozil treatment, suggesting an improvement of the **insulin** resistance index in this patient subgroup. Gemfibrozil treatment did not affect plasma fibrinolysis or fibrinogen levels, despite marked reduction of plasma **triglycerides** and improvement of the **insulin** sensitivity associated with **triglyceride** normalization. Copyright (C) 2000 Elsevier Science Ireland Ltd.

L85 ANSWER 13 OF 53 CAPLUS COPYRIGHT 2001 ACS
1999:219995 Document No. 130:306599 Antisense oligonucleotides capable of binding to multiple targets and their use in the treatment of respiratory disease. Nyce, Jonathan W. (East Carolina University, USA). PCT Int. Appl. WO 9913886 A1 19990325, 120 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US19419 19980917. PRIORITY: US 1997-59160 19970917; US 1998-93972 19980609.
AB Antisense oligonucleotides carrying sequences that will allow them to bind to more than one mRNA in a target cell are described. Such oligonucleotides can be used as a single treatment for diseases having more than one contributing pathway. In particular, oligonucleotides effective against genes involved in the etiol. of respiratory disease are targeted. Preferably, the oligonucleotides are low in adenosine (.ltoreq.15%) and may have adenosines substituted with analogs. These oligonucleotides are targeted to high (G+C) sequences within mRNAs. Thus, phosphorothioate antisense oligonucleotide (HAdA1AS, 5'-gatggagggcggcatggcggg-3') designed for the adenosine A1 receptor is provided. HAdA1AS significantly and specifically reduces the in vivo response to adenosine challenge in a dose-dependent manner, is effective in protection against aeroallergen-induced bronchoconstriction (house dust

mite), has an unexpected long-term duration of effect (8.3 days for both PC50 adenosine and resistance), and is free of side effects that might be toxic to the recipient. Such oligonucleotides may be used for treating a disease or condition assocd. with lung airway, such as bronchoconstriction, inflammation, or allergies.

L85 ANSWER 14 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-183551 [17] WPIDS
AB CA 2270320 A UPAB: 20000405
NOVELTY - A microcapsule composition (I) comprising a biocompatible oil and a polymer capable of forming microspheres, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) the use of (I) to deliver an active agent to an animal;

(2) the use of (I) to increase the bioactivity of an active agent in an animal;

(3) the use of (I) to treat diabetes; and

(4) a method (II) for preparing a microsphere composition (i.e. (I)), comprising:

(a) adding a polymer capable of forming microspheres to a biocompatible oil;

(b) adding an active agent in an aqueous solution to the polymer and oil solution;

(c) pouring the mixture into a non-solvent solution under conditions that allow for the spontaneous formation of microspheres containing the naturally occurring oil and the active agent.

ACTIVITY - Antidiabetic.

D-ala2-GLP-1 microspheres were given to a mouse model of Type II diabetes to determine whether or not therapeutic levels GLP-1 could be delivered **orally** to diabetics. The mouse model used was the DB/DB mouse which had a leptin receptor mutation that inactivated the receptor. This mutation resulted in the mouse being hyperplagic, hyperglycemic, hyperinsulinemic and obese. The late onset of hyperglycemia and hyperinsulinemia in these mice was a similar characteristic found in type II diabetes. It was found that there was no difference seen between control mice and mice treated with D-ala2-GLP-1 microspheres immediately after administration (t=0 hours), as expected, however the response to **orally** administered microspheres at t=4 and 8 hours was a downward shift in both basal and stimulated glycemia as compared to the control mice. Therefore in addition to being able to deliver **orally** D-ala2-GLP-1 to non-diabetic mice, it was also possible to **orally** deliver therapeutic levels of the peptide over a 10 hour period. These results suggested that **oral** delivery of peptides may be useful in the treatment of type II diabetes and other disorders requiring the continuous presence of a peptide.

MECHANISM OF ACTION - The presence of the biocompatible oil increases the release of active agents from the microspheres. GLP-1 stimulates glucose dependent **insulin** release and possibly improves peripheral **insulin** sensitivity.

USE - (I) may be used to deliver an active agent (e.g. hormones, **proteins**, peptides, peptide analogs, peptide derivatives, drugs, vaccines, **antigens**, vitamins, **carbohydrates** and/or **lipids**) to an animal, to increase the bioavailabilty of an active

agent in an animal and to treat diabetes (if the active agent used is GLP-1 (glucagon-like peptide-1)) (claimed).

ADVANTAGE - The presence of the biocompatible oil increases the release of active agents from the microspheres. Also, GLP-1 appears to be unable to cause hypoglycemia even at high peptide concentrations.

Dwg.0/11

L85 ANSWER 15 OF 53 MEDLINE DUPLICATE 4

1999250318 Document Number: 99250318. Dietary **lipids** modify the cytokine response to bacterial lipopolysaccharide in mice. Sadeghi S; Wallace F A; Calder P C. (Institute of Human Nutrition, University of Southampton, Southampton, UK.) IMMUNOLOGY, (1999 Mar) 96 (3) 404-10. Journal code: GH7. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom.

Language: English.

AB To investigate the effect of dietary **lipids** with different **fatty acid** compositions upon the in vivo cytokine response to bacterial lipopolysaccharide (LPS), mice were fed for 5 weeks on a low-fat diet or on one of four high-fat diets that contained 20%, by weight, of coconut oil (CO), **olive oil** (OO), **safflower oil** (SO) or fish oil (FO). The mice were **injected** intraperitoneally with a non-lethal dose of **Escherichia coli** LPS (100 micrograms/20 g body weight) and killed 90 or 180 min later. Plasma tumour necrosis factor-alpha (TNF-alpha), interleukin (IL)-1alpha, IL-6 and IL-10 concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Plasma TNF-alpha and IL-10 concentrations were higher 90 min postinjection than after 180 min, whereas plasma IL-1beta and IL-6 concentrations were higher 180 min postinjection than after 90 min. Peak plasma TNF-alpha, IL-1beta and IL-6 concentrations were lower in the CO- and FO-fed mice than in those fed the SO diet. Peak plasma IL-10 concentrations were higher in CO-fed mice than in those fed some of the other diets. These observations suggest that, relative to the n-6 polyunsaturated **fatty acid**-rich SO diet, CO and FO diminish production of proinflammatory cytokines in vivo. This indicates that these **fatty acids** might be useful therapies in acute and chronic inflammatory diseases. The enhanced production of IL-10 following CO feeding appears to be an additional antiinflammatory effect of this oil, which could give added benefit in various clinical conditions.

L85 ANSWER 16 OF 53 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5

2000:203745 Document No.: PREV200000203745. Diabetes, **insulin** resistance and dyslipidaemia in lipodystrophic HIV-infected patients on highly active antiretroviral therapy (HAART. Vigouroux, C.; Gharakhanian, S.; Salhi, Y.; Nguyen, T.-H.; Chevenne, D.; Capeau, J. (1); Rozenbaum, W..

(1) INSERM U.402, Faculte de Medecine Saint-Antoine, 27 Rue Chaligny, 75571, Paris, Cedex 12 France. Diabetes & Metabolism, (Sept., 1999) Vol. 25, No. 3, pp. 225-232. ISSN: 1262-3636. Language: English. Summary Language: English; French.

AB This study assessed glucose tolerance, **insulin** sensitivity and **lipid** parameters in HIV-infected patients presenting with lipodystrophy during HAART including protease inhibitors. Fourteen consecutive patients from Rothschild Hospital treated with HAART and presenting with marked facial lipoatrophy were evaluated. A 75 g

oral glucose tolerance test (OGTT) with measurement of plasma glucose, **insulin**, proinsulin and free **fatty acids** at T0, 30, 60, 90 and 120 min was performed. **Lipid** parameters (**triglycerides**, cholesterol, apolipoproteins A1 and B) were studied as well as nutritional and inflammatory markers (albumin, prealbumin, transferrin, haptoglobin, orosomucoid, C-reactive **protein**), endocrine and cytokine parameters (thyrotropin, cortisol, leptin, interleukin-6), HIV viral load and CD4-lymphocyte count.

These patients were compared with 20 non-lipodystrophic protease inhibitor-treated patients. The measurements performed during OGTT showed that among the 14 lipodystrophic patients, 11 (79%) presented with diabetes (5 patients) or normal glucose tolerance but with **insulin** resistance (6 patients). This frequency was strikingly different in the group of non-lipodystrophic patients, which included only 4 (20%) presenting with diabetes (1 patient), or impaired glucose tolerance (2 patients), or normal glucose tolerance but with **insulin** resistance (1 patient). Hypertriglyceridaemia was present in 11 lipodystrophic (79%) versus 7 non-lipodystrophic patients (35%). Nutritional and endocrine measurements were normal. an abnormal processing of proinsulin to **insulin** was excluded. Thus, lipodystrophy during HAART was associated with diabetes, **insulin** resistance and hypertriglyceridaemia. Diabetes, diagnosed by basal and/or 120 min-OGTT glycaemia, seems more frequent than previously described. The therapeutic consequences of these results deserve evaluation in clinical trials.

L85 ANSWER 17 OF 53 MEDLINE

1999011479 Document Number: 99011479. A comparison of biodegradable microparticles and MF59 as systemic adjuvants for recombinant gD from HSV-2. Singh M; Carlson J R; Briones M; Ugozzoli M; Kazzaz J; Barackman J; Ott G; O'Hagan D. (Adjuvant Research Division, Chiron Corporation, Emeryville, CA 94608, USA.. manmohan_singh@cc.chiron.com) . VACCINE, (1998 Nov) 16 (19) 1822-7. Journal code: X6O. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A recombinant form of glycoprotein D from herpes simplex virus type-2 (gD2) was encapsulated into polylactide-co-glycolide (PLG) microparticles using a previously established solvent evaporation technique. The mean size of the microparticles was about 1 micron and high encapsulation efficiency of the **antigen** was achieved (70-80%). The microparticles were administered intramuscularly to Balb/C mice and the immune responses were compared with those obtained with the **oil** in **water** adjuvant MF59. The serum IgG response to gD2 induced by the microparticles was comparable with that induced by MF59. The serum neutralization titres were also comparable for microparticles and the emulsion. However, the microparticles induced a higher IgG2a isotype response and a more potent serum IFN-gamma response than MF59, suggesting a more Th1 type of response. The MF59 induced higher levels of serum IL-4 and IL-5 cytokines, suggesting a more Th2 type of response.

L85 ANSWER 18 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

1998368434 EMBASE Effects of fish oil alone and combined with long chain (n-6) **fatty acids** on some coronary risk factors in

male subjects. Haglund O.; Wallin R.; Wretling S.; Hultberg B.; Saldeen T.. Prof. T. Saldeen, Department of Forensice Medicine, University of Uppsala, Dag Hammarskjolds vag 17, S-752 37 Uppsala, Sweden. Journal of Nutritional Biochemistry 9/11 (629-635) 1998.
Refs: 37.
ISSN: 0955-2863. CODEN: JNBIEL.
Publisher Ident.: S 0955-2863(98)00065-5. Pub. Country: United States.
Language: English. Summary Language: English.

AB The effects of fish oil alone were compared with those of fish oil combined 1:1 with evening primrose oil rich in long chain (n-6) **fatty acids** in a double-blind cross-over study. After administration of fish oil there was a highly significant increase in 20:5 (n-3) (eicosapentaenoic acid) and 22:6 (n-3) (docosahexaenoic acid) and a significant decrease in (n-6) **fatty acids** in plasma phospholipids. After consumption of the fish oil/evening primrose oil mixture, the increase in (n-3) and the decrease in (n-6) **fatty acids** were considerably smaller. **Triglycerides** in serum decreased by 36% ($P < 0.01$) after the fish oil and by 29% ($P < 0.05$) after the fish oil/evening primrose oil mixture. Atherogenic index decreased by 12% ($P < 0.05$) after fish oil/evening primrose oil and by 6% (P = ns) after fish oil alone. This difference was statistically significant ($P < 0.05$). Plasma homocysteine was reduced by 10% ($P < 0.05$) after the fish oil/evening primrose oil mixture and decreased 4% (P = ns) after the fish oil alone. Plasma fibrinogen decreased after both oils. The combined oils did not raise plasminogen activator inhibitor-1 (PAI-1) **antigen** at all, whereas after fish oil there was a 49% ($P < 0.05$) increase. Fish oil increased the ratio C20:4 to C20:3, an index of delta-5-desaturase, by 96% ($P < 0.001$) and reduced the ratio of C20:3 to C18:2, an index of delta-6-desaturase, by 38% ($P < 0.001$), whereas the fish oil/evening primrose oil mixture left these indexes unchanged. A high index of delta-5-desaturase has been found to be correlated to increased **insulin** sensitivity. In conclusion, combination of fish oil and evening primrose oil had a more favorable effect on the atherogenic index and caused no increase in PAI-1 **antigen**. The effects on **triglycerides** and PAI-1 of the fish oil and the mixture appears to be a result of their (n-3) **fatty acid** content.

L85 ANSWER 19 OF 53 MEDLINE
97394103 Document Number: 97394103. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal **injection** of lipopolysaccharide. Webel D M; Finck B N; Baker D H; Johnson R W. (Department of Animal Sciences, University of Illinois, Urbana 61801, USA.) JOURNAL OF ANIMAL SCIENCE, (1997 Jun) 75 (6) 1514-20.
Journal code: HC7. ISSN: 0021-8812. Pub. country: United States.
Language:
English.

AB The emerging view is that reduced feed intake, lean muscle accretion, and growth in immunologically challenged pigs is the result of increased cytokine activity, but this has not been directly tested. To begin addressing this issue, 72 crossbred barrows and gilts (11.55 +/- .19 kg BW) were not fed for 12 h and then **injected** i.p. with 0, .5, or

5 micrograms/kg of **Escherichia coli** lipopolysaccharide (LPS). Blood was collected by jugular puncture at 0, 2, 4, 8, 12, and 24 h after **injection**. Plasma levels of **tumor necrosis** factor-alpha (TNF-alpha), interleukin-6 (IL-6), cortisol, plasma urea nitrogen (PUN), NEFA, and **triglycerides** were determined. Immunological stress was induced by LPS as indicated by increased secretion of TNF-alpha, IL-6, and cortisol. In pigs receiving 5 micrograms/kg of LPS, plasma TNF-alpha was increased 10-fold at 2 h after **injection** and was still elevated ($P < .01$) at 4 h. In these same pigs, plasma concentration of IL-6 was increased at 2 h and peaked at 4 h with levels exceeding baseline values by 200-fold ($P < .01$). Cortisol was elevated at 2, 4, and 8 h after **injection** ($P < .01$). The increased secretion of cytokines and cortisol in pigs **injected** with 5 micrograms/kg of LPS was followed by an increase in **protein** degradation, as evidenced by PUN values that were increased two- and threefold at 8 and 12 h after **injection**, respectively. However, unlike previous reports in laboratory animal species, plasma glucose, NEFA, and **triglycerides** were not altered by LPS. Nonetheless, as the period of feed deprivation progressed from 12 to 36 h, plasma NEFA and **triglycerides** increased ($P < .05$) and plasma glucose tended to decrease. We believe that immunological challenge induces cytokine synthesis and secretion in swine which, in turn, may induce **protein** catabolism.

L85 ANSWER 20 OF 53 MEDLINE DUPLICATE 6
97387044 Document Number: 97387044. Short-term oestrogen replacement therapy improves **insulin** resistance, **lipids** and fibrinolysis in postmenopausal women with NIDDM. Brussaard H E; Gevers Leuven J A; Frolich M; Kluft C; Krans H M. (Department of Endocrinology and Metabolic Diseases, University Hospital, Leiden, The Netherlands.) DIABETOLOGIA, (1997 Jul) 40 (7) 843-9. Journal code: E93. ISSN: 0012-186X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Oestrogen replacement therapy is associated with a decreased risk of cardiovascular disease in postmenopausal women. Patients with non-**insulin**-dependent diabetes mellitus (NIDDM) have an increased cardiovascular risk. However, oestrogen replacement therapy is only reluctantly prescribed for patients with NIDDM. In a double blind randomized placebo controlled trial we assessed the effect of **oral** 17 beta-estradiol during 6 weeks in 40 postmenopausal women with NIDDM. Glycated haemoglobin (HbA1c), **insulin** sensitivity, suppressibility of hepatic glucose production, lipoprotein profile and parameters of fibrinolysis were determined. The oestrogen treated group demonstrated a significant decrease of HbA1c and in the normotriglyceridaemic group a significantly increased suppression of hepatic glucose production by **insulin**. Whole body glucose uptake and concentrations of non-esterified **fatty acids** did not change. LDL-cholesterol- and apolipoprotein B levels decreased, and HDL-cholesterol, its subfraction HDL2-cholesterol and apolipotrotein A1 increased. The plasma **triglyceride** level remained similar in both groups. Both the concentration of plasminogen activator inhibitor-1 **antigen** and its active subfraction decreased. Tissue type plasminogen activator activity increased significantly only in the normotriglyceridaemic group. Oestrogen replacement therapy improves

insulin sensitivity in liver, glycaemic control, lipoprotein profile and fibrinolysis in postmenopausal women with NIDDM. For a definite answer as to whether oestrogens can be more liberally used in NIDDM patients, long term studies including the effect of progestogens are necessary.

L85 ANSWER 21 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
97173401 EMBASE Document No.: 1997173401. Dietary fish oil enhances circulating **interferon**-.gamma. in mice during listeriosis without altering in vitro production of this cytokine. Fritsche K.L.; Feng C.; Berg J.N.. Dr. K.L. Fritsche, 110 Animal Sciences Research Center, University of Missouri, Columbia, MO 65211, United States. Journal of Interferon and Cytokine Research 17/5 (271-277) 1997.
Refs: 56.
ISSN: 1079-9907. CODEN: JICRFJ. Pub. Country: United States. Language: English. Summary Language: English.
AB The objective of this study was to investigate the impact of feeding mice a diet rich in n-3 polyunsaturated **fatty acids** (PUFA) from fish oil on the **interferon**-.gamma. (IFN-.gamma.) response during an active infection with Listeria monocytogenes. Weanling female C3H/Hen mice were fed experimental diets containing 20% by weight one of the following fats: **soybean oil**, lard, or a mixture of menhaden fish oil and **corn oil** (17:3, w/w). After 4 weeks, mice were **injected** with 105 live L. monocytogenes, and the concentration of IFN- .gamma. in serum and spleen was determined 0, 2, 4, and 7 days postinfection by enzyme-linked immunosorbent assay (ELISA). Fish oilfed mice showed significantly higher IFN-.gamma. in their blood at 2 and 4 days postchallenge compared with mice fed the **soybean oil**-containing or lard-containing diets ($p < 0.001$). A higher concentration of IFN-.gamma. was also found in the spleen homogenate of fish oil-fed mice on day 4 postchallenge ($p < 0.005$). To examine in vitro IFN-.gamma. production, splenocytes were isolated from fish oil- fed and **soybean oil**-fed mice on day 4 postchallenge and cultured with concanavalin A (1 .mu.g/ml and 10 .mu.g/ml) for 24 and 48 h. There were no significant differences in the IFN-.gamma. concentration in cell culture supernatants between these diet treatments. This study demonstrated that the elevation in the concentration of IFN-.gamma. in blood and spleen during murine listeriosis is accentuated and prolonged by dietary n-3 PUFA, and these effects may not be due to changes in IFN-.gamma. production.

L85 ANSWER 22 OF 53 MEDLINE DUPLICATE 7
97430749 Document Number: 97430749. Effects of troglitazone on **insulin** action and cardiovascular risk factors in patients with non-**insulin**-dependent diabetes. Sironi A M; Vichi S; Gastaldelli A; Pecori N; Anichini R; Foot E; Seghieri G; Ferrannini E. (Metabolism Unit, CNR Institute of Clinical Physiology, Pisa, Italy.) CLINICAL PHARMACOLOGY AND THERAPEUTICS, (1997 Aug) 62 (2) 194-202. Journal code: DHR. ISSN: 0009-9236. Pub. country: United States. Language: English.
AB OBJECTIVE: **Insulin** resistance is a potential target for pharmacologic intervention in non-**insulin**-dependent diabetes.

Troglitazone is being evaluated as an **insulin** enhancer in **insulin** resistant states. RESEARCH DESIGN AND METHODS: We randomized 40 patients with non-**insulin**-dependent diabetes to diet plus placebo (n = 15) or diet plus troglitazone (n = 25; 200 mg/day) treatment for 8 weeks. Fasting endogenous glucose production (EGP, by the stable isotope technique) and whole-body **insulin** sensitivity (by the **insulin** suppression test) were measured at baseline and on days 3, 7, 14, 28, and 56 of treatment. RESULTS: By day 56, fasting plasma glucose had risen from 12.0 +/- 0.9 to 12.8 +/- 1.2 mmol/L in the placebo group and had fallen from 12.4 +/- 0.6 to 11.3 +/- 0.6 mmol/L in the troglitazone group (p = 0.03). This was the result of small improvements in whole-body **insulin** sensitivity (steady-state plasma glucose during the **insulin** suppression test: from 11.09 +/- 1.1 to 10.3 +/- 0.8 mmol/L versus 13.8 +/- 1.0 to 10.0 +/- 0.9 mmol/L, placebo versus troglitazone; p = 0.01) and EGP (from 103% +/- 3% versus 96% +/- 2% of baseline, placebo versus troglitazone; p = 0.09). The time course of **insulin** action showed an early (first week of treatment) decrease in EGP in the troglitazone group that was maintained throughout, whereas steady-state plasma glucose levels began to diverge toward the end of treatment. The effects of **insulin** on plasma free **fatty acid** and potassium concentrations were not different between placebo and troglitazone. The cardiovascular risk profile (heart rate; serum **triglycerides**; total, low-density lipoprotein, and high-density lipoprotein cholesterol; proinsulin; uric acid; plasminogen activator inhibitor-1 **antigen** and activity; 24-hour blood pressure monitoring and urinary albumin excretion) was unaltered by troglitazone treatment. CONCLUSIONS: Troglitazone as monotherapy for typical non-**insulin**-dependent diabetes had a modest anti-hyperglycemic effect and, at the dose used in this study, had no effect on cardiovascular risk factors.

L85 ANSWER 23 OF 53 CAPLUS COPYRIGHT 2001 ACS
1996:440899 Document No. 125:96040 Immunogenic compositions solubilised in a hydrophobic solvent. New, Roger Randal Charles (Cortecs Limited, UK). PCT Int. Appl. WO 9614871 A1 19960523, 30 pp. DESIGNATED STATES: W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-GB2675 19951114. PRIORITY: GB 1994-22990 19941115.

AB An immunogenic compn. comprising an immunogen solubilised, or otherwise distributed, in a hydrophobic solvent in the absence of a hydrophilic phase. Preferably, the immunogenic compn. is provided as an **oral** vaccine. Thus, 40 .mu.L of tetanus toxoid (5 mg/mL) was added to 1 mL **dispersion** of 100 mg/mL soya phosphatidyl choline and the mixt. was lyophilized overnight, followed by addn. of 1 mL of oleic acid to obtain a crystal clear soln. Mice were administered 100 .mu.L of above soln. either s.c. or through an intragastric tube. Antibody levels against tetanus **antigen** after two wk was much more than controls.

L85 ANSWER 24 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
96211483 EMBASE Document No.: 1996211483. Attenuated GLP-1 secretion in

obesity: Cause or consequence?. Ranganath L.R.; Beety J.M.; Morgan L.M.; Wright J.W.; Howland R.; Marks V.. School of Biological Sciences, University of Surrey,Guildford, Surrey GU2 5XH, United Kingdom. Gut 38/6 (916-919) 1996.
ISSN: 0017-5749. CODEN: GUTTAK. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Background - Hypersecretion of insulinotropic factors such as glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(7-36)amide (GLP-1) have been postulated to account for the hyperinsulinaemia of obesity. Aims - To examine the role of GLP-1 and GIP in obese women and matched controls. Subjects - Six lean and six obese women subjects matched for age. Methods - The gut hormone, plasma glucose, and serum **triglyceride** responses were studied over 180 minutes after **oral carbohydrate** and fat meals. Heparin (10 000 units) was given intravenously at 120 minutes. Results - There was pronounced attenuation of plasma GLP-1 secretion to **oral carbohydrate** in the obese compared with lean subjects but no such difference in response to **oral** fat load. There were no differences in the plasma GIP responses to **carbohydrate** or fat feeding. There was an apparent fall in plasma GLP-1 values in all subjects after administration of heparin. Conclusion - Postprandial GLP-1 secretion in response to **oral carbohydrate** is considerably attenuated in obese subjects. The cause of this attenuation of GLP-1 secretion is not known although we suggest that both this fall and the overall reduction in GLP-1 values in obese subjects may be related to an increase in plasma non-esterified **fatty acids**.

L85 ANSWER 25 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1995-240478 [31] WPIDS
CR 1995-240479 [31]

AB WO 9517209 A UPAB: 20000818
A vaccine compsn. (I) comprises an **antigen** (Ag) or antigenic compsn. in association with an **oil-in-water** emulsion having the following compsn.: a metabolisable oil, such as **squalene**, alpha -tocopherol and Tween 80. The compsn. may opt. comprise QS21 and 3 De-O-acylated monophosphoryl **lipid** (3D-MPL).

USE - (I) is used in the mfr. of a vaccine for the prophylactic or immunotherapeutic treatment of viral, bacterial, parasitic infections or cancer. (I) are potent inducers of a range of immune responses. For human admin. QS21 and 3D-MPL will be present in the vaccine in the range 10-50 mu g. The **oil-in-water** will comprise 2-10% **squalene**, 2-10% alpha tocopherol and 0.3-3% Tween 80.

ADVANTAGE - The combination of the two adjuvants QS21 and 3D-MPL together with an **oil-in-water** emulsion can overcome the serious limitations of vaccines based on recombinant **proteins** and induce a wider spectrum of immune responses. In certain systems, the combination of 3D-MPL and QS21 together with an **oil-in-water** emulsion have been able to synergistically enhance **interferon** gamma prodn.
Dwg.0/4

L85 ANSWER 26 OF 53 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1995-082031 [11] WPIDS
AB WO 9503823 A UPAB: 19950322
A solid formulation for admin. of a therapeutic polypeptide (I) comprises 70-90 wt.% sodium salicylate and 10-30 (pref. 15-25) wt.% of an oil.

The oil is pref. selected from **mineral oil**, silicone oil, **corn oil**, **peanut oil**, coconut oil, **sesame oil**, **olive oil**, **fatty acids** and vitamin E, esp. peanut or **corn oil**.

USE - (I) is esp. human growth hormone or **insulin** (both claimed). Several other (I) are listed in the disclosure, e.g. vaccines, **antigens**, antibodies, TSH, renin, calcitonin, FSH, LHRH, oxytocin, vasopressin, **interferons**, interleukins and TNF.

ADVANTAGE - The Na salicylate/oil mixt. is a permeation enhancer which promotes absorption of (I) through the wall of the gastrointestinal tract, esp. of the colon, after **oral** admin. The solid formulation is easily handled and stored, and provides homogeneous **oral** dosage forms (e.g. pills, capsules and delivery vessels). The above ratio of Na salicylate to oil provides optimal absorption and bioavailability in a solid formulation. (I) may be non-lyophilised.
Dwg.1/5
ABEQ US 5424289 A UPAB: 19950727
Dosage form comprises on **orally** administable enteric-coated capsules contg. (a) human growth hormone or **insulin**; (b) 70-90 wt.% sodium salicylate; and (c) 10-30wt.% oil.

Opt. (a) is non-lyophilised. Formulation comprises a homogeneous mixt. of ingredients.

ADVANTAGE - Bioavatability of peptide through the colonic membrane of the gastrointestinal tract is 40% or more.
Dwg.0/6

L85 ANSWER 27 OF 53 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8
1994:646793 Document No. 121:246793 Effects of metformin and metoprolol CR on hormones and fibrinolytic variables during a hyperinsulinemic, euglycemic clamp in man. Landin, Kerstin; Tengborn, Lilian; Smith, Ulf (Department of Medicine, University of Goeteborg, Goeteborg, Swed.). Thromb. Haemostasis, 71(6), 783-7 (English) 1994. CODEN: THHADQ. ISSN: 0340-6245.
AB The aim of this study was to characterize the acute effect of euglycemic (glucose 5.2 mmol/L) hyperinsulinemia (mean 118 mU/L) on fibrinolytic variables, free **fatty acids** (FFA) and counterregulatory hormones in humans. In addn., the effect of chronic treatment with metformin, an **oral** antidiabetic agent which enhances **insulin** action, and metoprolol CR, a relatively .beta.1-selective adrenergic antagonist, was also evaluated. A randomized, double-blind, placebo-controlled, cross-over study including 18 non-obese men, aged 53 .+-. 6 yr, was performed. The investigations were performed after each treatment period of 6 wk in both the postabsorptive state and during a euglycemic, hyperinsulinemic clamp. Compared to the postabsorptive state, plasminogen activator inhibitor (PAI-1) activity and **antigen**, **tissue plasminogen activator** (t-PA) **antigen** and FFA decreased after 120 min of euglycemic hyperinsulinemia. In addn., t-PA activity increased while blood levels of lipoprotein (a) [Lp(a)],

catecholamines and cortisol remained unchanged. Growth hormone increased during the clamps and this was most pronounced after treatment with metoprolol CR. When the effect of treatment was compared, postabsorptive levels of C-peptide, FFA and t-PA **antigen** were lower after metformin than after the placebo period. The t-PA **antigen** also remained lower during the clamp after metformin treatment. No significant effects of metformin or metoprolol CR were seen on **insulin** -stimulated glucose uptake during the clamps or on postabsorptive levels of counterregulatory hormones, PAI-1 or Lp(a). Thus, the rapidly increased fibrinolytic activity after 2 h hyperinsulinemia with maintained euglycemia can not be explained by the concomitant changes in counterregulatory hormones. It is more likely that the decreased FFA and/or **triglyceride** levels play a role.

L85 ANSWER 28 OF 53 MEDLINE DUPLICATE 9

96428166 Document Number: 96428166. Metabolic, endocrine and haematological responses to intravenous E. coli endotoxin administration in 1-week-old calves. Kinsbergen M; Bruckmaier R M; Blum J W. (Division of Nutritional Pathology, University of Bern, Switzerland.) ZENTRALBLATT FUR VETERINARMEDIZIN. REIHE A, (1994 Sep) 41 (7) 530-47. Journal code: Y70. ISSN: 0514-7158. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Responses to i.v. **injected** E. coli endotoxin (E), followed by saline infusion, as compared with saline infusion alone, were studied for 24 h in 1-week-old calves. After administration of E, respiratory rate (RR), heart rate (HR), rectal temperature (RT), serum iron, **insulin**, (I), cortisol and **tumor necrosis** factor-alpha, transiently, and urea, continuously, increased. Isoleucine and leucine became elevated at 24 h, whereas white-blood-cell number, free **fatty acids** (FFA) and **triglycerides** (TG) increased after an initial fall. After administration of E, packed-cell volume, erythrocyte number, haemoglobin, glucose (G), cholesterol, phospholipids (PL), lysine, arginine, proline, citrulline, calcium (Ca), inorganic phosphorus, **insulin**-like growth factor I (IGF-I) and 3,5,3'-triiodothyronine (T3) concentrations and alkaline phosphatase (AP) and gamma-glutamyl transferase (gamma GT) activities increased significantly while growth hormone decreased non-significantly. When saline was infused alone, G, TG, PL, Ca, AP, gamma GT, I, IGF-I and T3 decreased, while FFA, urea and sodium increased, but, changes of G, urea, AP, IGF-I and T3 were less marked than after **injection** of E. Potassium, total **protein** and albumin concentrations, and glutamyl dehydrogenase and glutamate oxalacetate transaminase activities were not significantly affected by either treatment. In conclusion, metabolic and endocrine changes during saline infusion alone were typical for food withdrawal. Changes of variables after administration of E were transient, biphasic or sustained, thus expressing complex interactions between metabolic parameters, endocrine factors and cytokines.

L85 ANSWER 29 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

94185955 EMBASE Document No.: 1994185955. Metformin and metoprolol CR treatment in non-obese men. Landin K.; Tengborn L.; Smith U.. Department of Medicine, Sahlgrenska Hospital,S-413 45 Goteborg, Sweden. Journal of Internal Medicine 235/4 (335-341) 1994.

ISSN: 0954-6820. CODEN: JINMEO. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Objective. To study the effect of metformin and metoprolol CR on **insulin** sensitivity, blood **lipids**, fibrinolytic activity and blood pressure. Design. A double-blind, placebo controlled, triple cross-over study with randomization to either metformin, 850 mg b.i.d., or metoprolol CR 100 mg o.d., or placebo for a period of 18 weeks. The glucose uptake was measured with the euglycaemic clamp technique after every 6 weeks' treatment period. Blood pressure and blood samples were taken every 3rd week. Subjects. Eighteen non-obese men (53 .+-. 6 years of age). Results. Metformin decreased C-peptide ($P < 0.02$), FFA ($P < 0.003$), total and low-density lipoprotein cholesterol, **tissue plasminogen activator antigen** and the urinary potassium excretion ($P < 0.05$ for all), but not blood pressure compared to placebo. Metoprolol CR reduced diastolic blood pressure and pulse rate; fasting free **fatty acids** and the urinary potassium increased ($P < 0.05$ for all). No effect of metformin or metoprolol CR was seen on the glucose disposal rate, blood glucose, plasma **insulin**, **triglycerides**, high-density lipoprotein cholesterol, lipoprotein(a), uric acid or plasminogen activator inhibitor 1 activity or **antigen**. The glucose uptake was not particularly decreased in these subjects. Conclusion. The study shows that metformin has some favourable effects on metabolism and that metoprolol CR is fairly neutral in this regard. The lack of effect of metformin on glucose disposal rate and blood pressure can be explained by the fact that the individuals studied were neither **insulin** resistant nor hypertensive. The data does not preclude an antihypertensive effect by treating a concomitant **insulin** resistance.

L85 ANSWER 30 OF 53 MEDLINE DUPLICATE 10

95044276 Document Number: 95044276. Hemostasis profile and **lipid** metabolism with long-interval use of a desogestrel-containing **oral** contraceptive. Cachrimanidou A C; Hellberg D; Nilsson S; von Schoulz B; Crona N; Siegbahn A. (Department of Obstetrics and Gynecology, Falu Hospital, Falun, Sweden.) CONTRACEPTION, (1994 Aug) 50 (2) 153-65. Journal code: DQN. ISSN: 0010-7824. Pub. country: United States. Language: English.

AB Thirty healthy women, aged 18 to 37, were randomly allocated to treatment with a desogestrel-containing **oral** contraceptive, with 20 women using a nine-week on, one-week off regimen, and 10 women using the traditional regimen. At 0, 3 and 12 months, blood samples were drawn for liver **proteins**, lipoproteins and hemostatic variables. No significant changes were observed between the two regimens. Sex hormone binding globulin (SHBG) and corticosteroid binding globulin (CBG, transcortin) were increased two- to four-fold, as a measure of estrogenicity. Minimal changes occurred within the lipoprotein cholesterol fractions, whereas there were some increases within the lipoprotein **triglyceride** fractions. Among the hemostatic variables, there were significant increases of fibrinogen, factor VII and thrombin/antithrombin III (TAT) complex. The coagulation inhibitors, antithrombin III,

protein C and **protein** S, were essentially unchanged. A decrease of both **tissue plasminogen activator antigen** (t-PA) and **tissue plasminogen activator** inhibitor activity (PAI activity) of the fibrinolytic system was observed. A nine-week regimen does not seem to alter **lipid** metabolism and coagulation more than a three-week regimen.

L85 ANSWER 31 OF 53 MEDLINE DUPLICATE 11
94338416 Document Number: 94338416. Relationships of plasminogen activator inhibitor-1 to anthropometry, serum **insulin**, **triglycerides** and adipose tissue **fatty acids** in healthy men. Cigolini M; Targher G; Seidell J C; Schiavon R; Manara F; Zenti M G; Mattioli C; De Sandre G. (Institute of Clinical Medicine, University of Verona, Italy..) ATHEROSCLEROSIS, (1994 Apr) 106 (2) 139-47. Journal code: 95X. ISSN: 0021-9150. Pub. country: Ireland. Language: English.

AB Increased plasma levels of plasminogen activator inhibitor-1 (PAI-1), responsible for reduced fibrinolytic activity, have been shown to be an important risk factor for cardiovascular disease. PAI-1 plasma levels are influenced by several factors which have not yet been fully clarified, including dietary fat intake. The relationships of PAI-1 with other cardiovascular risk factors are still not well known. In a random sample of 38-year-old healthy men (n = 94), the association of PAI-1 plasma levels (measured as activity and **antigen**) with anthropometric parameters, serum **lipids**, fasting and 2 h **insulin** and glucose concentration after **oral** glucose-load was analysed. Furthermore, the **fatty acid** composition of subcutaneous adipose tissue, as an objective and reliable index of dietary fat intake, was measured. The univariate analysis showed that plasma levels of PAI-1 were significantly associated with body mass index (BMI) ($r = 0.37$, $P < 0.001$), waist/hip ratio (WHR) ($r = 0.26$, $P < 0.01$), serum **triglycerides** ($r = 0.47$, $P < 0.0001$), HDL/total cholesterol ratio ($r = -0.35$, $P < 0.001$), fasting and 2-h **insulin** ($r = 0.27$, $P < 0.01$ and $r = 0.34$, $P < 0.001$) and glucose concentrations ($r = 0.25$, $P < 0.05$ and $r = 0.28$, $P < 0.01$).(ABSTRACT TRUNCATED AT 250 WORDS)

L85 ANSWER 32 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
94096098 EMBASE Document No.: 1994096098. Long-term influence of omega-3 **fatty acids** on fibrinolysis, fibrinogen, and serum **lipids**. Eritsland J.; Seljeflot I.; Abdelnoor M.; Arnesen H.. Department of Cardiology, Ulleval Hospital,0407 Oslo, Norway. Fibrinolysis 8/2 (120-125) 1994.
ISSN: 0268-9499. CODEN: FBRIE7. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The long-term influence of omega-3 **fatty acids** (.omega.-3 FA) on fibrinolytic parameters before and after venous occlusion (VO), fibrinogen, and serum **lipids** was assessed in a group of moderately **hypertriglyceridemic** patients undergoing coronary artery bypass surgery. The patients (n = 58) were investigated before the operation (baseline), and 6 months afterwards. Following randomization postoperatively, 29 patients received 4 g .omega.-3 FA concentrate, containing 3.4 g eicosapentaenoic and docosahexaenoic acid per day. The remaining 29 patients constituted the control group. Plasminogen activator inhibitor (PAI-1) activity had decreased in both

groups at 6 months, whereas PAI-1 **antigen** increased in the .omega.-3 FA group and decreased in the control group (median change from baseline, 10.0% vs -12.9 %, p = 0.039). In both groups combined, there was a correlation between the change in PAI-1 **antigen** and the change in serum phospholipid .omega.-3 **fatty acids** (r = 0.28, p = 0.036). There was no group difference in **tissue plasminogen activator** activity and **antigen**, before as well as after VO. Global fibrinolytic activity after VO, assessed by the serum D-dimer method, was not statistically different in the two groups. Serum **triglyceride** levels were more reduced in the .omega.-3 FA group (median change from baseline, -34.5 % vs -11.1% in the control group, p = 0.001). There was no group difference in the concentrations of fibrinogen or cholesterol in lipoprotein fractions. Thus, an expected **triglyceride**-lowering effect by .omega.-3 FA was recorded, but except for an increase in PAI-1 **antigen**, no long-term influence on fibrinolytic parameters was noted.

L85 ANSWER 33 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

94247234 EMBASE Document No.: 1994247234. Dietary fish oil enhances macrophage production of nitric oxide. Chaet M.S.; Garcia V.F.; Arya G.; Ziegler M.M.. Division Pediatric Surgery, Department of Surgery, University of Cincinnati,Cincinnati, OH 45229, United States. Journal of Surgical Research 57/1 (65-68) 1994.
ISSN: 0022-4804. CODEN: JSGRA2. Pub. Country: United States. Language: English. Summary Language: English.

AB Nitric oxide (NO) is recognized as an important mediator of hemodynamic regulation and multisystem organ failure (MOF). Although polyunsaturated **fatty acids** (PUFA) are known to modify the elaboration of some humoral mediators in MOF, their effect upon NO production has not been evaluated. This study was designed to examine the effect of omega-3 (.omega.3) and omega-6 (.omega.6) PUFA on macrophage production of NO, TNF, PGE2, and PGI2. Rats were fed diets of 18% by calorie **safflower oil** (.omega.6) or fish oil (.omega.3) for 12 days. Bronchoalveolar macrophages (BAM) were divided into group A (medium only), group B (0.5 .mu.g/ml PGE2 or PGI2 + medium) or group C (10 .mu.M indomethacin + medium). Cells were stimulated with 100 U/ml **interferon-**.gamma. and 10 .mu.g/ml **Escherichia coli**-LPS. In group A, BAM from animals fed .omega.3 produced significantly more NO (3.64 vs 1.92 .mu.M, P < 0.05) and TNF (8.52 vs 1.75 .mu.g/ml, P < 0.05) than BAM from .omega.6-fed animals. The addition of exogenous PGE2 or PGI2 (group B) ablated the difference in NO and TNF observed in group A. Indomethacin also (group C) ablated the difference in NO and TNF production seen in .omega.3- and .omega.6-fed animals noted in group A. These data demonstrate that PUFA influence BAM production of NO and TNF. Changes in the .omega.6-derived prostanoids may account for the differences in TNF production, but these data suggest that PGE2 and PGI2 are not responsible for the observed differences in NO production. Through its effect upon macrophage NO production, manipulation of exogenous .omega.3/.omega.6 PUFA may be of value in the management of patients at risk for development of MOF.

L85 ANSWER 34 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

93351119 EMBASE Document No.: 1993351119. Effects of fish oil supplemented with pyridoxine and folic acid on homocysteine, atherogenic index, fibrinogen and plasminogen activator inhibitor-1 in man. Haglund O.; Hamfelt A.; Hambraeus L.; Saldeen T.. Department of Forensic Medicine, University of Uppsala, Sundsvalls Hospital,Sundsvall, Sweden. Nutrition Research 13/12 (1351-1365) 1993.
ISSN: 0271-5317. CODEN: NTRSDC. Pub. Country: United States. Language: English. Summary Language: English.

AB Twelve volunteers, healthy or with slightly to moderately increased blood **lipids**, participated in a double-blind cross-over study in which they received 30 mL of fish oil with or without supplementation of the B vitamins pyridoxine (80 mg/daily) and folic acid (10 mg/daily) for 4 weeks. These vitamins have been reported to decrease homocysteine, a risk factor for cardiovascular disease, and to be involved in the metabolism of unsaturated **fatty acids**. Fish oil with and without vitamin supplementation resulted in lower levels of **triglycerides** and increased HDL cholesterol levels. Atherogenic index decreased by 24% ($p<0.05$) after the vitamin supplemented fish oil and by 12% ($p<0.05$) after the other oil. Plasma fibrinogen decreased by 15% ($p<0.01$) after the vitamin supplemented fish oil and by 6% (n.s.) after the other fish oil. The effect on atherogenic index and fibrinogen were significantly different with the two treatments ($p<0.05$). Plasma homocysteine decreased by 30% after the vitamin supplemented oil, an effect due mainly to the vitamins. Plasminogen activator inhibitor-1 **antigen** increased by 68% ($p<0.01$) after fish oil alone and by 43% (n.s.) after the vitamin supplemented fish oil. The difference between the two treatments was not statistically significant. The fish oil **fatty acids** and these B vitamins might thus have a synergistic effect on the **lipid** metabolism and fibrinogen synthesis. The results may be of importance when fish oils are prescribed to persons with risk factors for cardiovascular disease.

L85 ANSWER 35 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

93211940 EMBASE Document No.: 1993211940. The effects of a biological response modifier, OK-432, on tumor-induced alterations in the host metabolism. Noguchi Y.; Tsuburaya A.; Makino T.; Fukuzawa K.; Nomura K.; Yoshikawa T.; Matsumoto A.; Moriya Y.; Masuda K.. First Department of Surgery, Yokohama City University, School of Medicine, 3-9 Fukuura,Kanazawa-ku, Yokohama 236, Japan. Surgery Today 23/7 (621-625) 1993.
ISSN: 0941-1291. CODEN: SUTOE5. Pub. Country: Japan. Language: English. Summary Language: English.

AB The effects of multicytokine inducer, OK-432, on tumor-induced metabolic alterations were studied by assessing three key regulatory enzymes of gluconeogenesis, de novo **fatty acid** synthesis and the **triglyceride** clearance pathways. Two Klinish Einheit (KE) of OK-432 was subcutaneously **injected** on alternate days, for 10 days, into Fischer 344 rats with or without methylcholanthrene-induced sarcoma. At the time of sacrifice, the tumors accounted for approximately 23% of their total body weight. The **injections** of OK-432 did not affect the amount of food intake in either the tumor bearers or the controls. The tissue lipoprotein lipase activities in the epididymal fat pads of the tumor bearers were significantly decreased compared with the controls ($P < 0.01$). Phosphoenolpyruvate carboxykinase activity in the

liver was significantly increased ($P < 0.01$), while malic enzyme activity tended to be decreased in the tumor bearers compared with the controls. However, there were no significant differences in those activities depending on the OK-432 **injections**, even though OK-432 induced **tumor necrosis** factor (TNF) and increased cytotoxic activities in the mesenteric lymph nodes as well as in the spleen. Thus, although the role of monokines in inducing cancer cachexia is not yet clearly understood, OK-432 was not able to revert the tumor-induced metabolic alterations which lead to tissue wasting and cancer cachexia.

L85 ANSWER 36 OF 53 CAPLUS COPYRIGHT 2001 ACS
1993:3112 Document No. 118:3112 Polyunsaturated **fatty acids** and experimental radioimmunotherapy of breast cancer. Blank, Edward W.; Lin, Chu Y.; Peterson, Jerry A.; Ceriani, Roberto L. (John Muir Cancer Aging Res. Inst., Walnut Creek, CA, 94596, USA). Antibody, Immunoconjugates, Radiopharm., 5(3), 307-16 (English) 1992. CODEN: AIRAEB. ISSN: 0892-7049.

AB Fed a dietary source of fat from either fish oil (MaxEPA), **corn oil**, or lard, BALB/c (nu/nu) nude mice carrying established transplantable human breast tumors (MX-1) were treated with either an unconjugated mixt. (cocktail) or an 131I-labeled cocktail of four (Mc1, Mc3, Mc5 and Mc8) anti-human milk fat globule **monoclonal antibodies** (MoAbs). Dietary manipulation was confirmed by **fatty acid** anal. of serum, liver and tumor samples. According to flow cytometry and biodistribution studies, no changes in target **antigen** content or uptake of the radiolabeled antibodies by the tumors was obsd. for any of the diets. Tumor vol. of mice fed MaxEPA supplemented diet was reduced to 36% of that of **corn oil** and lard fed control groups. However, **injection** of unconjugated MoAbs reduced tumor vols. only in **corn oil** fed nude mice, when each group was compared to its resp. control. In contrast, **injection** of 131I-MoAbs produced large tumor vol. redns. in all dietary groups. However, groups fed MaxEPA and **injected** with 131I-labeled MoAbs showed the greatest tumor redn. and enhanced therapeutic effect, without apparent toxic effects.

L85 ANSWER 37 OF 53 CAPLUS COPYRIGHT 2001 ACS
1991:614855 Document No. 115:214855 Sustained-release composition for macromolecular **proteins**. Sivaramakrishnan, Kallidaikuric; Gray, Matthew W. (Pitman-Moore, Inc., USA). PCT Int. Appl. WO 9105548 A1 19910502, 40 pp. DESIGNATED STATES: W: AU, BB, BG, BR, CA, FI, HU, JP, KP, KR, LK, MC, MG, MW, NO, RO, SD, SU; RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US5345 19900920. PRIORITY: US 1989-420156 19891010.

AB A sustained-release pharmaceutical compn. comprises a solid **wax** matrix having a macromol. **protein** and a water-insol. **surfactant** uniformly **dispersed** therein. A sustained release compn. contained beeswax 90, Mazol (I) 10, and bovine serum albumin 10% released 100% of the **protein** after 14 days as compared to 25% for the control having PPG instead of I.

L85 ANSWER 38 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
92049236 EMBASE Document No.: 1992049236. The use of alternative therapies by HIV-positive patients attending the St. Louis AIDS clinical trials unit.

Rowlands C.; Powderly W.G.. Aids Clinical Trials Unit, Box 8011, Washington University School of Medicine, 660 S. Euclid, St. Louis, MS 63110, United States. Missouri Medicine 88/12 (807-810) 1991. ISSN: 0026-6620. CODEN: MIMIA2. Pub. Country: United States. Language: English. Summary Language: English.

L85 ANSWER 39 OF 53 MEDLINE

92191257 Document Number: 92191257. Impact of transfer from animal-source **insulins** to biosynthetic human **insulin** (rDNA E coli) in patients with diabetes mellitus. Garber A J; Davidson J A; Krosnick A; Beaser R S; Anderson J H Jr. (Department of Medicine, Baylor College of Medicine, Houston, Texas..) CLINICAL THERAPEUTICS, (1991 Sep-Oct) 13 (5) 627-36. Journal code: CPE. ISSN: 0149-2918. Pub. country: United States. Language: English.

AB Six hundred forty-eight patients (50.5% men; 49.5% women) with diabetes mellitus on animal-source **insulin** therapy for at least five years were studied. In this patient population, approximately 68.7% had Type I **insulin**-dependent diabetes mellitus and 31.3% had Type II noninsulin-dependent diabetes mellitus, nonetheless requiring **insulin** therapy. Patients were voluntarily transferred from animal-source **insulin** to biosynthetic human **insulin** derived by recombinant DNA technology from genetically altered **Escherichia coli** [human **insulin** (rDNAE coli)] and were monitored regularly thereafter. At a mean interval of 14 months after transfer to human **insulin** (rDNAE coli), these patients had gained 0.8 kg in body weight (P less than 0.01). There was a significant decline in systolic (P less than 0.01) but not in diastolic blood pressure. **Insulin** requirements while on animal-source **insulin** averaged 47.6 +/- 22.9 U/day (mean +/- SD); this requirement was not significantly different after transfer to human **insulin** (rDNAE coli) (47.0 +/- 21.2 U/day). The distribution of regular and modified **insulin** types prescribed did not change after patients were transferred from animal-source **insulin** to human **insulin** (rDNAE coli). However, a significant increase in the number of **insulin injections** from 1.79 +/- 0.59 to 1.96 +/- 0.61 **injections**/day was observed (P less than 0.001). Fasting glucose levels declined significantly from 202 +/- 87 mg/dl on animal-source **insulin** to 178 +/- 66 mg/dl on human **insulin** (rDNAE coli) (P less than 0.001). Postprandial glucose levels (at two hours) also declined from 227 +/- 83 mg/dl to 212 +/- 80 mg/dl. Glycosylated hemoglobin (HbA1c) decreased from 9.57 +/- 2.01% while taking animal **insulin** to 8.97 +/- 2.00% on human **insulin** (rDNAE coli) (P less than 0.001) Serum cholesterol and **triglyceride** levels **insulin** (rDNAE coli). Serum high-density lipoprotein cholesterol (HDL-cholesterol) levels increased from 54.2 +/- 15.1 mg/dl on animal **insulin** to 57.2 +/- 15.5 mg/dl on human **insulin** (rDNAE coli) (P less than 0.001). These data demonstrate that transfer of patients from animal-source **insulins** to human **insulin** (rDNAE coli) was associated with: (1) an improvement in glycemic control parameters; (2) a slight increase in the number of **insulin injections** in some patients, but no overall alteration in **insulin** requirements; and (3) no adverse trends in indicators of cardiovascular risks, such as serum **lipids**. Indeed, overall cardiovascular risk may have declined not

only as a result of improvement in glycemic control, but also owing to a reduction in systolic blood pressure and an elevation in HDL-cholesterol levels.

L85 ANSWER 40 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
91266027 EMBASE Document No.: 1991266027. Gastrointestinal lymphatic absorption of peptides and **proteins**. Rubas W.; Grass G.M.. Genentech, Inc., Pharmaceut. Res./Development, 460 Point San Bruno Boulevard,South San Francisco, CA 94080, United States. Advanced Drug Delivery Reviews 7/1 (15-69) 1991.
ISSN: 0169-409X. CODEN: ADDREP. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB There is no doubt that intact peptides and **proteins** do cross the gastrointestinal wall into the lymphatics. Transfer from the lumen into the lymph system occurs in both lymphoid (PP) and non-lymphoid tissue (villous). Contribution by the paracellular pathway may be low. Transfer into lymph vessels via non-lymphoid tissue depends upon the **lipid** pathway, vehicle effects, sieving mechanisms of the blood vessels, and the application site. The best lymphatic access has been achieved from the proximal small intestine, while rectal application has also been proven to be suitable. Utilizing formulations composed of a long chain and unsaturated **fatty acid** in combination with a **surfactant** favors transfer into lymph. The most promising results were achieved with combinations resembling chylomicrons, attempting to direct the compound into chylomicrons. For smaller substances such as peptides, the physicochemical characteristics are one of the key factors for lymphatic uptake. Substances which are highly lipophilic favor lymphatic passage. Assessment of solubility in **peanut oil** and/or in the viscous isotropic phase of the digested **lipids** is a useful tool to predict the lymph absorption potential. In order to utilize the sieving mechanism, conversion of a substance into a drug-polymer complex such as dextran or cyclodextran together with co-application of an absorption promoter (bifunctional system) has been shown to be feasible and suitable for lymphatic delivery. Endocytotic processes if present at all play a minor role in non-lymphoid tissue uptake. The most prominent uptake mechanism for particles and microspheres in lymphoid tissue is phagocytosis. The extent depends on surface property, the amount administered, and the suspension vehicle. Hydrophobic surfaces and aqueous suspending vehicles appear best. Transcytosis through PP, also called the M-cell route, seems to be most suited for highly potent compounds such as lymphokines and **antigens** (vaccines). The reasons are: (a) limited number of PP, thus, the overall surface area is relatively small, and therefore the total absorption potential is limited, and (b) PP tissue is rich in lymphocytes, thus, substances which interact with lymphocytes are best targeted to PP when using the **oral** route. **Oral** delivery to local lymph nodes by means of carrier systems (i.e. poly(lactide-co-glycolide) microspheres) via the M-cell route appears very promising. Migration, however, into and through the mesenteric lymph appears limited to microspheres less than 5 .mu.m in diameter. Though both cell types, M cells and enterocytes, share the same common glycoproteins and glycolipids a number of microorganisms are able

to bind selectively to a receptor on the M-cell surface and thereby enter the host. Utilizing the microorganism's ligand could be beneficial for specific targeting to PP, bypassing lysosomal degradation in absorptive cells. Moreover, transport of a membrane-bound macromolecule by M cells is about 50 times more efficient than a soluble, non-adherent macromolecules.

L85 ANSWER 41 OF 53 CAPLUS COPYRIGHT 2001 ACS
1991:178380 Document No. 114:178380 Enhancing cancer therapy by administration of unsaturated **fatty acids**. Ceriani, Roberto Luis; Peterson, Jerry Arthur (USA). Eur. Pat. Appl. EP 385859 A1 19900905, 29 pp. DESIGNATED STATES: R: DE, ES, FR, GB, IT, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1990-400552 19900228. PRIORITY: US 1989-317411 19890301.
AB Polyunsatd. **fatty acids**, prostaglandins, prostacyclins, thromboxanes, leukotrienes, malonaldehyde and triene epoxides enhance the effectiveness of cancer therapy, such as by chemotherapeutic agents, hormones, external or internal irradn., anti-target cell antibodies, biol.-response modifiers, anti-cell **antigen** specific antibodies and hyperthermia. The vol. of transplanted MX-1 breast tumors in mice was decreased by the **injection** of 131I-conjugated anti-breast epithelial **monoclonal antibodies**. The effect was enhanced when the mice were kept on a diet of fish-type oil, rich in polyunsatd. **fatty acids**, such as eicosapentaenoic and docosahexaenoic acid. **Corn oil** had only a slight effect.

L85 ANSWER 42 OF 53 CAPLUS COPYRIGHT 2001 ACS
1990:513539 Document No. 113:113539 Lipopolysaccharide and **tumor necrosis** factor cause a fall in plasma concentration of **lecithin**: cholesterol acyltransferase in cynomolgus monkeys. Ettinger, Walter H.; Miller, Lisa D.; Albers, John J.; Smith, Thuy K.; Parks, John S. (Dep. Intern. Med., Wake Forest Univ., Winstom-Salem, NC, 27103, USA). J. Lipid Res., 31(6), 1099-107 (English) 1990. CODEN: JLPRAW. ISSN: 0022-2275.
AB The effects of i.v. **injection** of lipopolysaccharide (LPS) and **tumor necrosis** factor alpha (TNF) were investigated in cynomolgus monkeys (Macaca fascicularis). **Injection** of 20 .mu.g/kg of LPS from **Escherichia coli** (serotype 055:B5) into cynomolgus monkeys fed a monkey chow diet caused a 2-fold increase in plasma **triglyceride** and a 25% redn. in plasma cholesterol 48 h after **injection**. Similar results were found with **injection** of recombinant human TNF at a dose of 20 .mu.g/kg into chow-fed animals. However, **injection** of the same dose of LPS or TNF into animals fed an atherogenic diet contg. satd. fat and cholesterol resulted in a 2.4-5-fold increase in plasma **triglyceride** concns. and no significant change in plasma cholesterol levels. The fall in plasma cholesterol levels obsd. in chow-fed animals was assocd. with a 57% decrease in the cholesteryl ester (CE) content in low d. lipoprotein (LDL) and 35% decrease in CE in high d. lipoprotein (HDL) in LPS-**injected** animals, and a decrease of 33% in CE concn. of LDL and 41% in CE of HDL in animals **injected** with TNF. In animals fed the atherogenic diet contg. satd. fat and

cholesterol, the **injection** of both LPS and TNF also resulted in a significant decrease in the CE content of LDL and HDL. However, the plasma total cholesterol levels did not change in the animals fed satd. fat and cholesterol because the decrease in CE content of LDL and HDL was offset by an increase in very low d. lipoprotein (VLDL)-CE. Plasma **lecithin**:cholesterol acyltransferase (LCAT) activity declined to 22% and 54% of control values 24 h after LPS and TNF **injection**, resp. Similarly, plasma concn. of LCAT mass detd. by RIA declined 35% in animals **injected** with LPS and 27% in animals **injected** with TNF within 24 h. Direct addn. of LPS or TNF to an in vitro assay of LCAT activity in control plasma had no effect on LCAT activity suggesting that LPS and TNF did not directly inhibit LCAT activity. A part of the lipoprotein changes obsd. after LPS **injection** may be due to a decline in LCAT mass and a decrease in generation of cholesteryl ester in plasma. The cytokine, TNF, is an important mediator of these phenomena.

L85 ANSWER 43 OF 53 MEDLINE DUPLICATE 12

90240355 Document Number: 90240355. **Triglyceride** kinetics, tissue lipoprotein lipase, and liver lipogenesis in septic rats. Lanza-Jacoby S; Tabares A. (Department of Surgery, Jefferson Medical College, Philadelphia, Pennsylvania 19107.) AMERICAN JOURNAL OF PHYSIOLOGY, (1990 Apr) 258 (4 Pt 1) E678-85. Journal code: 3U8. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB The mechanism for the development of **hypertriglyceridemia** during gram-negative sepsis was studied by examining liver production and clearance of very-low-density lipoprotein (VLDL) **triglyceride** (TG). To assess liver output and peripheral clearance the kinetics of VLDL-TG were determined by a constant iv infusion of [2-3H]glycerol-labeled VLDL. Clearance of VLDL-TG was also evaluated by measuring activities of lipoprotein lipase (LPL) in heart, soleus muscle, and adipose tissue from fasted control, fasted E. coli-treated, fed control, and fed E. coli-treated rats. Lewis inbred rats, 275-300 g, were made septic with 8 x 10(7) live E. coli colonies per 100 g body wt. Twenty-four hours after E. coli **injection**, serum TG, free **fatty acids** (FFA), and cholesterol of fasted E. coli-treated rats were elevated by 170, 76, and 16%, respectively. The elevation of serum TG may be attributed to the 67% decrease in clearance rate of VLDL-TG in fasted E. coli-treated rats compared with their fasted controls. The suppressed activities of LPL in adipose tissue, skeletal muscle, and heart were consistent with reduced clearance of TG. Secretion of VLDL-TG declined by 31% in livers of fasted E. coli-treated rats, which was accompanied by a twofold increase in the composition of liver TG. Rates of in vivo TG synthesis in livers of the fasted E. coli-treated rats were twofold higher than in those of fasted control rats. Decreased rate of TG appearance along with the increase in liver synthesis of TG contributed to the elevation of liver **lipids** in the fasted E. coli-treated rats.(ABSTRACT TRUNCATED AT 250 WORDS)

L85 ANSWER 44 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

91150982 EMBASE Document No.: 1991150982. The comparative effects of n-3 and n-6 polyunsaturated **fatty acids** on plasma fibrinogen levels: A controlled clinical trial in **hypertriglyceridemic** subjects. Radack K.; Deck C.; Huster G.. Division of General Internal Medicine, 231 Bethesda Avenue, Mail Location 535, Cincinnati, OH 45267,

United States. Journal of the American College of Nutrition 9/4 (352-357)
1990.
ISSN: 0731-5724. CODEN: JONUDL. Pub. Country: United States. Language: English. Summary Language: English.

AB It has previously been shown that fish oil supplementation, compared to **olive oil**, reduces plasma fibrinogen. Presented here are the results of a randomized, double-blind, crossover controlled trial that compared the effects of dietary n-3 and n-6 **fatty acid** supplementation on plasma fibrinogen levels in 10 patients with hyperlipoproteinemia types IIb or IV. Plasma fibrinogen levels showed statistically significant reductions during both the fish oil and **corn oil** treatment periods. Other variables related to hemostasis which showed no significant changes from baseline included **tissue plasminogen activator** activity and inhibitor, **protein** C **antigen**, antithrombin III activity, bleeding time, and platelet counts. These data confirm the two previous reports that fish oil supplementation is associated with reductions in plasma fibrinogen levels, thereby modifying a potential nonlipid risk factor for cardiovascular disease. Unlike previous reports, however, n-6 polyunsaturated **fatty acids** were also associated with significant reductions in fibrinogen levels. Therefore, it is premature to conclude that the fibrinogen-lowering effects of dietary fish oil are unique to n-3 polyunsaturated **fatty acids**
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L85 ANSWER 45 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

89171034 EMBASE Document No.: 1989171034. Plasma fibrinolytic activity after ingestion of omega-3 **fatty acids** in human subjects.
Takimoto G.; Galang J.; Lee G.K.; Bradlow B.A.. Division of Hematology, Department of Pathology, Michael Reese Hospital and Medical Center, Chicago, IL, United States. Thrombosis Research 54/6 (573-582) 1989.
ISSN: 0049-3848. CODEN: THBRAA. Pub. Country: United States. Language: English. Summary Language: English.

AB Plasma fibrinolytic activity was measured in human volunteers after 30 day periods of ingestion of fish oil product (Max Vita) containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and a wheat germ oil product containing alpha linolenic acid. Compliance was confirmed by significant increases in plasma levels of EPA and DHA and by significant falls in serum **triglyceride** levels. Platelet aggregation in platelet rich plasma and in whole blood was not altered significantly by fish oil or wheat germ oil. Neither fish oil or wheat germ oil caused any significant change in **tissue plasminogen activator** (tPA) or its inhibitor (PAI) measured enzymatically or in tPA **antigen** measured by an ELISA method. All these analytes (tPA, PAI, and tPA **antigen**) were measured before and after venous compression.

L85 ANSWER 46 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

88265642 EMBASE Document No.: 1988265642. Reduction in plasminogen activator inhibitor-1 (PAI-1) with omega-3 polyunsaturated **fatty acid** (PUFA) intake. Mehta J.; Lawson D.; Saldeen T.. Veterans

Administration Medical Center, Gainesville, FL, United States. American Heart Journal 116/5 I (1201-1206) 1988.
ISSN: 0002-8703. CODEN: AHJOA2. Pub. Country: United States. Language: English. Summary Language: English.

AB Activity of plasminogen activator inhibitor-1 (PAI-1) in human blood correlates with thrombotic tendency and with serum **triglyceride** concentrations. Since intake of fish-derived omega-3 polyunsaturated **fatty acids** (PUFA) decreases serum **triglycerides**, we examined the effects of omega-3 PUFA maximum eicosapentaenoic acid (Max EPA) intake on PAI-1 levels in eight patients with coronary artery disease and in four normal subjects. After 4 weeks of Max-EPA intake by coronary artery disease patients, serum **triglyceride** concentrations and PAI-1 levels decreased 43 .+-. 8% and 21 .+-. 5%, respectively (both p .ltoreq. 0.01) without any change in **tissue plasminogen activator** (TPA) levels. No changes were noted at 1 week of Max EPA intake in normal subjects, but at 3 weeks serum **triglyceride** concentrations and PAI-1 levels decreased 32 .+-. 13% and 22 .+-. 4%, respectively (p .ltoreq. 0.02) without change in TPA. The magnitude of reduction in **triglycerides** was dependent on the initial serum concentration (r = 0.68, p .ltoreq. 0.01). In addition, decrease in PAI-1 levels correlated with reduction in serum **triglycerides** (r = 0.79, p .ltoreq. 0.01). This study shows that omega-3 PUFA intake reduces PAI-1 levels without change in TPA **antigen**. These observations may relate to decrease in thrombotic activity upon consumption of large amounts of fish or fish-derived products.

L85 ANSWER 47 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

78374618 EMBASE Document No.: 1978374618. Glucose tolerance in viral **hepatitis**. A study of twenty patients during the acute phase and after recovery. Chupin M.; Charbonnel B.; Le Bodic L.; et al.. Dept. Clin. Med. B, CHU, Hotel-Dieu, 44035-Nantes, France. Diabetes 27/6 (661-669) 1978.
CODEN: DIAEAZ. Pub. Country: United States. Language: English.

AB Twenty patients with viral **hepatitis** were investigated during the acute phase and after recovery in order to study their glucose tolerance. During **hepatitis**, three tests were performed: **oral** and intravenous glucose tolerance tests and tolbutamide test, with measurements of glucose, **insulin**, free **fatty acids**, **triglycerides**, growth hormone, and cortisol. After recovery, a control **oral** glucose tolerance test was performed. All these results were compared with those of healthy controls.
During the acute phase, both **oral** and intravenous glucose tolerance tests showed two groups of patients: nine with normal glucose tolerance but significant hyperinsulinism, and 11 with glucose intolerance but a delayed and not increased **insulin** response. Tolbutamide test was always normal, without any difference between these two groups. Free **fatty acids** (FFA) were increased only in the glucose-intolerant group; there was a significant negative correlation between basal FFA and the K values. **Triglycerides** were increased in the two groups, but were higher in the normal glucose-tolerant one. Growth hormone and cortisol were increased only when the K value was very

low. All these parameters returned to normal in all the patients, after their recovery. Clinical features, hepatic tests, and HLA typing were similar in the two groups, with no significant differences. A comparative group of patients with mild and nonhepatic infections did not show any abnormalities. This study suggests there is a transitory **insulin** -resistant state during viral **hepatitis**, which can be due to an increase in FFA concentrations. Glucose intolerance occurs in half of the cases, possibly as a consequence of an additional .beta.-cell impairment.

L85 ANSWER 48 OF 53 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 13
1978:577527 Document No. 89:177527 Changing circulating levels of **lipids**, **insulin** and glucagon during experimental acute pancreatitis. Mizuma, K.; DeLamater, P. V.; Lee, P. C.; Appert, H. E.; Howard, John M. (Dep. Surg., Med. Coll. Ohio, Toledo, Ohio, USA). Surg., Gynecol. Obstet., 147(4), 577-82 (English) 1978. CODEN: SGOBA9. ISSN: 0039-6087.

AB Acute pancreatitis was induced by intraductal **injection** of 10 mg/kg taurocholate or **Escherichia coli** endotoxin (1 mg/kg) in dogs. Plasma amylase and lipase levels rose significantly in both instances. In dogs receiving taurocholate, **insulin** and glucose levels in the plasma rose significantly 0.5-1 h after onset of pancreatitis. No change in plasma glucagon levels were obsd. In dogs receiving endotoxin, neither a transient increase in **insulin** nor a hyperglycemia was obsd. Instead, a rise in glucagon levels, 3 to 24 h after onset of pancreatitis, was seen. This was accompanied by hypoglycemia. Also, a rise in plasma free **fatty acids** and **triglyceride** levels was evident at 4 to 48 h after the onset of pancreatitis. I.m. **injection** of endotoxin did not result in an increase in serum amylase or lipase levels but caused hyperglucagonemia. No change in the plasma free **fatty acid** concn. was found in these dogs and only a short term change in the **triglyceride** level in the plasma was seen. Thus, pancreatitis, per se, does not necessarily result in an increase in plasma **insulin** levels. The 2 forms of induced pancreatitis produced strikingly different hormonal patterns. Plasma glucose levels did not always correspond to predicted hormonal levels, in this instance, hypoglycemia was accompanied by hyperglucagonemia and hyperglycemia by hyperinsulinism. Endotoxin may have a direct effect in causing hyperglucagonemia.

L85 ANSWER 49 OF 53 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 14
1977:173384 Document No.: BA63:68248. REINVESTIGATION OF HYPER GLYCEMIC RESPONSE TO GLUCAGON AND THE EFFECT OF GLUCAGON ON BLOOD CHOLESTEROL AND TRI GLYCERIDE CONCENTRATIONS PART 2 CHANGES IN LIVER DISEASE. OKUNO G; KURODA K. FOLIA ENDOCRINOL JPN, (1976 (RECD 1977)) 52 (12), 1202-1211. CODEN: NNGZAZ. ISSN: 0029-0661. Language: Unavailable.

AB Early preparations of glucagon were contaminated by considerable amounts of **insulin** and other impurities. This led to the reinvestigation of the hyperglycemic response using a recent glucagon preparation which contained very little **insulin** (5 mu[milliunits]/mg). Since little is known about the influence of glucagon on the cholesterol and **triglyceride** metabolism and because **carbohydrate** metabolism is closely related to **lipid** metabolism, blood concentrations of cholesterol and **triglyceride** were determined after glucagon administration. In the previous report, hyperglycemic

response to glucagon in diabetics was positively correlated with fasting blood glucose, which differed entirely from earlier reported results. Glucagon induced a statistically significant decrease in **triglyceride** concentration but no change in cholesterol concentration both in normal and diabetic. In this study, 34 cases with liver disease including 18 cases with acute **hepatitis**, 7 cases with chronic **hepatitis** and 9 cases with liver cirrhosis were investigated. Glucagon, 1 mg dissolved in 20 ml of physiological saline, was **injected** i.v., and blood specimens were analyzed before and 15, 30, 45, 60, 90 and 120 min after administration. The mean maximum increment of blood glucose in acute **hepatitis** (14.4 .+-. 2.3 mg/dl, mean .+-. SEM) and liver cirrhosis (22.9 .+-. 5.1 mg/dl) were significantly lower compared to normal controls (44.6 .+-. 4.3 mg/dl). The increment in chronic **hepatitis** (29.7 .+-. 6.3 mg/dl) decreased. In acute **hepatitis**, the maximum glucose response to glucagon correlated inversely with the degree of jaundice. The peak time of blood glucose was more delayed than in normals. In chronic **hepatitis** and liver cirrhosis, the return of blood glucose to basal levels was also more delayed than in normals. **Insulin** secretion after glucagon **injection** was almost abolished in acute **hepatitis**. In chronic liver disease, especially in cirrhosis of the liver, .SIGMA.IRI [total immunoreactive **insulin**] was higher than in normals. Significant improvement of both hyperglycemic and **insulin** response to glucagon were obtained in acute **hepatitis** after treatment. Blood **triglyceride** concentrations after glucagon were not constant in individual cases. The mean concentrations of **triglyceride** in acute and chronic **hepatitis** during the experiment showed a very slight and transient decrease and in liver cirrhosis, a little increase at the end of the experiment. No change was observed in blood cholesterol concentrations in the cases studied. These results clearly indicate a decrease of liver glycogen storage in liver disease and suggest that a glucagon tolerance test in liver disease might be a promising method for evaluating both glucose tolerance and liver function as 1 of the auxiliary tests.

L85 ANSWER 50 OF 53 MEDLINE

75170427 Document Number: 75170427. Hormonal and metabolic changes induced by elevated plasma free **fatty acids** in term pregnancy. I. Effect on maternal blood glucose, **insulin** and human placental lactogen circulating levels. Gaspard U J; Sandront H M; Luyckx A S; Lefebvre P J. JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (1975 Jun) 40 (6) 1066-72. Journal code: HRB. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB The influence of plasma free **fatty acid** (FFA) concentration on the secretion of human placental lactogen (hPL) was investigated in 16 normal young women during the last month of gestation, in order to determine whether hPL secretion is influenced in the same way as human growth hormone (hGH) during plasma FFA elevation. Maternal blood glucose (BG), plasma **triglycerides** (TG), FFA, immunoreactive **insulin** (IRI) and hPL levels were measured during and after a **lipid** emulsion infusion for 75 min (10 cases). The intravenous **injection** of 5,000 U of heparin at the 15th min of the **lipid** infusion was followed by a decrease in plasma **triglyceride** levels and by an accompanying rise in plasma FFA (rom

468 plus or minus 52 to 2,478 plus or minus 310 mueq/liter). In control experiments **lipid** infusion alone (3 cases) resulted in a moderate increase in FFA (718 plus or minus 157 to 1,046 plus or minus 255 mueq/liter), and separate iv heparin administration (3 cases) elevated the FFA levels from 728 plus or minus 50 to 1,649 plus or minus 153 mueq/liter). No significant change in either IRI or hPL levels was discernible in any of the tests performed. A tendency of blood glucose to increase was observed after heparin administration. It was concluded that a marked and sustained plasma FFA elevation, achieved through intravenous **lipid** and heparin infusion cannot alter hPL circulating levels in term human pregnancy.

L85 ANSWER 51 OF 53 MEDLINE
72266533 Document Number: 72266533. Effect of long-chain **triglyceride** on human **insulin** secretion. Carroll K F; Nestel P J. DIABETES, (1972 Sep) 21 (9) 923-9. Journal code: E8X. ISSN: 0012-1797. Pub. country: United States. Language: English.

L85 ANSWER 52 OF 53 CAPLUS COPYRIGHT 2001 ACS
1970:485525 Document No. 73:85525 **Carbohydrate** and **lipid** metabolism in viral **hepatitis**. Gioannini, Paolo; Gragnoli, Giorgio; Pupillo, Angelo; Scalise, Giorgio (Ist. Clin. Med. Gen. Ter. Med., Univ. Siena, Siena, Italy). Fegato, 16(1), 315-31 (Italian) 1970. CODEN: FGTOAW.
AB Clin. studies made on 21 patients with virus **hepatitis** were based on a comparative study of the glucemia, insulinemia, and plasma non-esterified **fatty acids** after an **oral** load of glucose. The glucemia was studied after an i.v. dose of glucose and after tolbutamide. The behavior of **triglycerides** and of cholesterol in the plasma was studied under basal conditions. These studies show the presence of alterations of the **carbohydrate** and **lipid** exchange, in the genesis of which factors antagonistic to **insulin** participate to an important extent.

L85 ANSWER 53 OF 53 CAPLUS COPYRIGHT 2001 ACS
1970:53489 Document No. 72:53489 Summit metabolism in young lambs. Effect of hormones and drugs that affect mobilization and utilization of substrate for cold-induced thermogenesis. Alexander, George (Div. Anim. Physiol., C.S.I.R.O., Prospect, Aust.). Biol. Neonatorum, 15(1), 37-48 (English) 1970. CODEN: BINEAA.
AB Various substances that affect mobilization and utilization of the major metabolic fuels, free **fatty acids**, and glucose, were given to lambs less than a week old and the effects on summit metabolism were examd. Short-term infusions of corticotropin, thyrotropin, glucagon, **insulin**, triiodothyronine, and hydrocortisone succinate failed to increase summit metabolism. Likewise glucagon and corticotropin failed to increase basal metabolism, though a thermogenic effect has been reported to occur in newborn rabbits. When triiodothyronine, cortisone acetate, or carnitine were given by i.m . **injection** over 2-4 days, only triiodothyronine produced a small increase in summit metabolism. Infusion

of an emulsion of **soybean oil** resulted in a small increase in summit metabolism, but caffeine was without effect. Summit metabolism was significantly reduced by bacterial endotoxin, but was not affected by dinitrophenol although this abolished shivering. The lack of response to infusion of the various hormones indicates that th eir natural secretion rate at the time of cold exposure does not limit the summit metabolic response of young lambs. However, the results suggest that summit metabolism could be influenced by the secretion rate of thyroid hormone during the few days preceding exposure to cold.

'IN' IS NOT A VALID FIELD CODE
L86 1560 FILE MEDLINE
L87 4987 FILE CAPLUS
L88 3249 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L89 1450 FILE EMBASE
L90 3688 FILE WPIDS
L91 560 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L92 15494 KIM M?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L93 5603 FILE MEDLINE
L94 16786 FILE CAPLUS
L95 11233 FILE BIOSIS
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L96 5340 FILE EMBASE
L97 13000 FILE WPIDS
L98 1827 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L99 53789 KIM S?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L100 107 FILE MEDLINE
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L105 62 FILE JICST-EPLUS

TOTAL FOR ALL FILES
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L108 16793 FILE CAPLUS
L109 9922 FILE BIOSIS

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L110 4313 FILE EMBASE
L111 17216 FILE WPIDS
L112 1872 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L113 54381 KIM J?/AU,IN

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L115 1 FILE CAPLUS
L116 0 FILE BIOSIS
L117 0 FILE EMBASE
L118 1 FILE WPIDS
L119 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L120 2 L113 AND L106 AND L99 AND L92

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L121 1 DUP REM L120 (1 DUPLICATE REMOVED)

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L121 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
2000:493356 Document No. 133:125288 Lipophilic microparticles containing a protein drug or antigen and formulation comprising same for controlled release. **Kim, Myung Jin**; Kim, Sun Jin; Kwon, Kyu Chan; Kim, Joon (LG Chemical Limited, S. Korea). PCT Int. Appl. WO 2000041682 A1 20000720, 49 pp. DESIGNATED STATES: W: AU, BG, BR, CA, CN, HU, ID, IL, IN, JP, MX, NZ, PL, SG, TR, US, ZA; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-KR25 20000114. PRIORITY: KR 1999-1232 19990118; KR 1999-59776 19991221.

AB A lipophilic microparticle having an av. particle size ranging from 0.1 to 200 .mu.m, comprising a lipophilic substance and an active ingredient selected from the group consisting of a protein or peptide drug and an antigen, retains the full activity of the active ingredient, and when formulated in the form of an oil dispersion or oil-in-water emulsion, it releases in an in vivo environment the active ingredient in a controlled manner over a long period.

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COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 483.77 483.92

DOCUMENT NUMBER: 98020882
TITLE: Microencapsulation of **antigens** using biodegradable polyesters: facts and phantasies.
AUTHOR: Kissel T; Koneberg R; Hilbert A K; Hungerer K D
CORPORATE SOURCE: Department of Pharmaceutics and Biopharmacy, Philipps-University, Marburg, Germany.
SOURCE: BEHRING INSTITUTE MITTEILUNGEN, (1997 Feb) (98) 172-83. Ref: 30
Journal code: 9KI. ISSN: 0301-0457.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104

AB New vaccination approaches and new **delivery** systems have been subject of intensive research activities recently. Controlled release vaccine **delivery** systems depend on the microencapsulation of **antigens** into biodegradable polymers, yielding small spherical polymeric particles, in the size range of 1-100 microns. By manipulating the micromorphology of the **microparticles** and degradation properties of the polymer either continuous or pulsatile release patterns can be adjusted. As biodegradable polymers mainly copolymers of lactic- and glycolic acid have been utilized, since these materials are known to be biocompatible and non-toxic. Apart from modulation of **antigen** release, an improvement of the adjuvant effect and an increase of in vitro (shelf-life) and in vivo stability of the **antigen** are issues of general interest with respect to parenteral vaccine **delivery** systems. Using different **microparticles** that release **antigens** in a pulsatile pattern at predetermined timepoints one hopes to induce protective immunity by a single administration of the vaccine **delivery** system. Using tetanus toxoid (TT) as a model **antigen** we have examined the stability during preparation, in vitro release and storage of TT **microparticles**. TT is a complex protein mixture sensitive to changes in pH conditions (pH < 5) and to thermal stress. TT **microparticles** can be prepared by a W/O/W double emulsion technique with satisfactory encapsulation efficiencies in good yields. In accordance with other investigators we observe an adjuvant effect of TT microspheres in mice upon sc administration leading to a **long-lasting** antibody response. In challenge experiments we could demonstrate a protective effect. The issue of an ideal release pattern remains open, since a boosting of the antibody titers during the bioerosion of the TT microspheres was not observed, possibly due to desactivation of TT in the degrading microspheres.

DOCUMENT NUMBER: PREV200100025097
TITLE: Biodegradable **microparticles** with different release profiles: Effect on the immune response after a single administration via intranasal and intramuscular routes.
AUTHOR(S): Spiers, Ian D.; Eyles, Jim E.; Baillie, Les W. J.; Williamson, E. Diane; Alpar, H. O. (1)
CORPORATE SOURCE: (1) Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET: H.O.Alpar@Aston.ac.uk UK
SOURCE: Journal of Pharmacy and Pharmacology, (October, 2000) Vol. 52, No. 10, pp. 1195-1201. print.
ISSN: 0022-3573.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In the development of single-dose microparticulate vaccines, identification of the type of protein release profile required to elicit high and sustainable immune responses is important. **Microparticles** exhibiting different protein release profiles (continuous, pulsatile and plateau) were made by solvent evaporation or solvent extraction methods from biodegradable polymers encapsulating the model **antigen**, bovine serum albumin (BSA). The immune responses obtained after a single intranasal or intramuscular administration of **microparticles** were determined, and also after a subcutaneous boost after 11 months. **Microparticles** were manufactured with acceptable protein loading and average volume size ranging from 1 to 10 mum. The integrity of BSA extracted and released from **microparticles** after 2 months incubation was retained. Microparticulate preparations administered by either intranasal or intramuscular routes, evoked rapid, high titre and long-lived (up to 11 months after priming) specific serum IgG responses which were significantly greater than for free BSA. The type of protein release from **microparticles** had no significant effect on the systemic immune responses. Interestingly, a formulation exhibiting a plateau-release profile was the only microparticulate system capable of inducing significantly greater IgA responses than free BSA after intranasal immunization. This study shows the benefit of microencapsulation in inducing high and **long-lasting** systemic immune responses after a single dose by both parenteral and mucosal **delivery**. We conclude that of the **microparticles** tested, the longevity and magnitude of humoral responses was not effected by the type of in-vitro protein release profile.

FILE 'MEDLINE, BIOSIS, EMBASE' ENTERED AT 09:18:20 ON 13 APR 2001

L1	0	S	LIPOPHILIC MICROPARTICLE
L2	4722	S	MICROPARTICLE
L3	35501	S	LIPOPHIL?
L4	40	S	L2 AND L3
L5	22409	S	HYALURONIC ACID?
L6	0	S	L4 AND L5
L7	25835	S	LECITHIN?
L8	0	S	L4 AND L7
L9	61161	S	AEROSOL
L10	0	S	L9 AND L4
L11	1217374	S	ANTIGEN?
L12	0	S	L11 AND L4
L13	414770	S	DELIVER?
L14	854	S	L13 AND L2
L15	186	S	L14 AND L11
L16	0	S	L15 AND L7
L17	0	S	L15 AND L9
L18	0	S	L17 AND L5
L19	41442	S	LONG LASTING
L20	7	S	L19 AND L15
L21	3	DUP REM	L20 (4 DUPLICATES REMOVED)
L22	280021	S	FATTY ACID?
L23	4	S	L22 AND L2 AND L13
L24	3	DUP REM	L23 (1 DUPLICATE REMOVED)
L25	47	S	L22 AND L5
L26	3	S	L25 AND L7
L27	1	DUP REM	L26 (2 DUPLICATES REMOVED)

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only as a result of improvement in glycemic control, but also owing to a reduction in systolic blood pressure and an elevation in HDL-cholesterol levels.

L85 ANSWER 40 OF 53 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
91266027 EMBASE Document No.: 1991266027. Gastrointestinal lymphatic absorption of peptides and **proteins**. Rubas W.; Grass G.M.. Genentech, Inc., Pharmaceut. Res./Development, 460 Point San Bruno Boulevard, South San Francisco, CA 94080, United States. Advanced Drug Delivery Reviews 7/1 (15-69) 1991.
ISSN: 0169-409X. CODEN: ADDREP. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB There is no doubt that intact peptides and **proteins** do cross the gastrointestinal wall into the lymphatics. Transfer from the lumen into the lymph system occurs in both lymphoid (PP) and non-lymphoid tissue (villous). Contribution by the paracellular pathway may be low. Transfer into lymph vessels via non-lymphoid tissue depends upon the **lipid** pathway, vehicle effects, sieving mechanisms of the blood vessels, and the application site. The best lymphatic access has been achieved from the proximal small intestine, while rectal application has also been proven to be suitable. Utilizing formulations composed of a long chain and unsaturated **fatty acid** in combination with a **surfactant** favors transfer into lymph. The most promising results were achieved with combinations resembling chylomicrons, attempting to direct the compound into chylomicrons. For smaller substances such as peptides, the physicochemical characteristics are one of the key factors for lymphatic uptake. Substances which are highly lipophilic favor lymphatic passage. Assessment of solubility in **peanut oil** and/or in the viscous isotropic phase of the digested **lipids** is a useful tool to predict the lymph absorption potential. In order to utilize the sieving mechanism, conversion of a substance into a drug-polymer complex such as dextran or cyclodextran together with co-application of an absorption promoter (bifunctional system) has been shown to be feasible and suitable for lymphatic delivery. Endocytotic processes if present at all play a minor role in non-lymphoid tissue uptake. The most prominent uptake mechanism for particles and microspheres in lymphoid tissue is phagocytosis. The extent depends on surface property, the amount administered, and the suspension vehicle. Hydrophobic surfaces and aqueous suspending vehicles appear best. Transcytosis through PP, also called the M-cell route, seems to be most suited for highly potent compounds such as lymphokines and **antigens** (vaccines). The reasons are: (a) limited number of PP, thus, the overall surface area is relatively small, and therefore the total absorption potential is limited, and (b) PP tissue is rich in lymphocytes, thus, substances which interact with lymphocytes are best targeted to PP when using the **oral** route. **Oral** delivery to local lymph nodes by means of carrier systems (i.e. poly(lactide-co-glycolide) microspheres) via the M-cell route appears very promising. Migration, however, into and through the mesenteric lymph appears limited to microspheres less than 5 .mu.m in diameter. Though both cell types, M cells and enterocytes, share the same common glycoproteins and glycolipids a number of microorganisms are able

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Gastrointestinal lymphatic absorption of peptides and proteins

Werner Rubas and George M. Grass*

Syntex Research, Institute of Pharmaceutical Sciences, Palo Alto, CA 94303, U.S.A.

(Received August 22, 1990)
(Revised manuscript received March 12, 1991)
(Accepted March 27, 1991)

Key words: Physiology of the gastrointestinal lymph system; Peyer's patch; Macromolecular uptake into the lymphatics; Strategy for oral lymph targeting; Site-specific absorption for lymphokines and antigens

Contents

Abbreviations: ASC, antibody-secreting cell; BLM, bleomycin; BSA, bovine serum albumin; CAP, cellulose acetate phthalate; CsA, cyclosporin A; DDT, 1,1-bis(*p*-chlorophenyl)-2;2,2-trichloroethane; DEMM, diethylmethylidenmalonate; FAE, follicle-associated epithelium; FITC, fluorescein isothiocyanate; GALT, gut-associated lymphoid tissue; GI, gastrointestinal; HBC, hexachlorobenzene; HCFU, 1-hexylcarbamoyl-5-fluorouracil; HEV, high-endothelial venule; HRP, horseradish peroxidase; i.d., intraduodenal; IFN, interferon; i.g., intragastric; IgA, immunoglobulin A; IgG, immunoglobulin G; IL, interleukin; i.p., intraperitoneal; LFA, lymphocyte function-associated antigen; MDP, muramyldipeptide; MHC, major histocompatibility complex; MM, mixed micelles; P_c, partition coefficient; PC, phosphatidylcholine; PCV, postcapillary venule; PEG, poly(ethylene glycol); PG, propylene glycol; PP, Peyer's patch; P_v, venous outflow pressure; SC, secretory component; σd, Staverman reflection coefficient; sIgA, secretory immunoglobulin A; SPF, specific pathogen-free; TJ, tight junction; TNF, tumor necrosis factor; VLDL, very-low-density lipoprotein; WGA, wheat germ agglutinin.

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Summary

There is no doubt that intact peptides and proteins do cross the gastrointestinal wall into the lymphatics. Transfer from the lumen into the lymph system occurs in both lymphoid (PP) and non-lymphoid tissue (villous). Contribution by the paracellular pathway may be low. Transfer into lymph vessels via non-lymphoid tissue depends upon the lipid pathway, vehicle effects, sieving mechanisms of the blood vessels, and the application site. The best lymphatic access has been achieved from the proximal small intestine, while rectal application has also been proven to be suitable. Utilizing formulations composed of a long chain and unsaturated fatty acid in combination with a surfactant favors transfer into lymph. The most promising results were achieved with combinations resembling chylomicrons, attempting to direct the compound into chylomicrons. For smaller substances such as peptides, the physicochemical characteristics are one of the key factors for lymphatic uptake. Substances which are highly lipophilic favor lymphatic passage. Assessment of solubility in peanut oil and/or in the viscous isotropic phase of the digested lipids is a useful tool to predict the lymph absorption potential. In order to utilize the sieving mechanism, conversion of a substance into a drug-polymer complex such as dextran or cyclodextran together with co-application of an absorption promoter (bifunctional system) has been shown to be feasible and suitable for lymphatic delivery. Endocytotic processes if present at all play a minor role in non-lymphoid tissue uptake. The most prominent uptake mechanism for particles and microspheres in lymphoid tissue is phagocytosis. The extent depends on surface property, the amount administered, and the suspension vehicle. Hydrophobic surfaces and aqueous suspending vehicles appear best. Transcytosis through PP, also called the M-cell route, seems to be most suited for highly potent compounds such as lymphokines and antigens (vaccines). The reasons are: (a) limited number of PP, thus, the

overall surface area is relatively small, and therefore the total absorption potential is limited, and (b) PP tissue is rich in lymphocytes, thus, substances which interact with lymphocytes are best targeted to PP when using the oral route. Oral delivery to local lymph nodes by means of carrier systems (i.e. poly(lactide-co-glycolide) microspheres) via the M-cell route appears very promising. Migration, however, into and through the mesenteric lymph appears limited to microspheres less than 5 μm in diameter. Though both cell types, M cells and enterocytes, share the same common glycoproteins and glycolipids a number of microorganisms are able to bind selectively to a receptor on the M-cell surface and thereby enter the host. Utilizing the microorganism's ligand could be beneficial for specific targeting to PP, bypassing lysosomal degradation in absorptive cells. Moreover, transport of a membrane-bound macromolecule by M cells is about 50 times more efficient than a soluble, non-adherent macromolecule.

I. Introduction

The gastrointestinal (GI) tract is certainly the route of choice for drug delivery. Many newly emerging drugs are peptides or proteins ranging in size from two up to several hundred amino acids [1]. Oral administration, however, is challenging because peptides/proteins are poorly absorbed, and are often unstable under the harsh gastrointestinal conditions. Furthermore, some are highly potent, and specifically active in a defined environment. For example, targeting cells of the immune system, i.e., lymph targeting, has the potential for stimulation, inhibition or modulation of virtually any part of the immune response for which distinct cell populations may be demonstrated to play a role. Delivery from the intestinal tract to the lymphatic system instead of the portal venous system may avoid extensive first-pass liver metabolism. Thus, a deeper understanding of the target site as well as physiologic events along the GI tract is necessary to develop optimally performing oral formulations successfully.

This review has as its primary goal a description of the gastrointestinal physiology relating to lymphoid and corresponding non-lymphoid tissues and identification of the requirements for improving strategies in GI lymph targeting. The extent of lymphatic transport depends on: (a) the physicochemical properties of the compound; (b) the permeability of the barriers over the lymphatic vessels; and (c) the composition of the formulation.

The lymph system includes the lymphatic vessels and the solitary, and aggregated lymphoid tissues such as tonsils, PP, appendix, diffuse collections of plasma cells and lymphocytes between epithelial cells covering the villi. It is now the general consensus that the aggregated lymphoid tissues are specialized but nonetheless peripheral tissues, wherein immune responses are initiated [2].

II. Absorption into lymphatic vessels via non-lymphoid tissue

II.1. Physiology of the lymphatic vessels

Lymphatic vessels of the small intestine originate as elongated, blind-ended ves-

sels (lacteals) in the center of each villus (Fig. 1). Finger-like villi such as those of man, dog, and cat host one central lacteal. However, in flattened villi, such as those found in rats, mice, and rabbits there are several vessels. The lacteals lie approximately 50 μm beneath the epithelium in a gel-like structure (interstitium) through which water and solutes slowly percolate, and their radius is about 10 μm (Fig. 2). These vessels join a network of lymphatic capillaries in the glandular layer of the mucosa and are linked to collecting lymphatics. At the mesenteric border the lymphatics leave the intestine in association with blood vessels [3–6]. In contrast to the small intestine, large intestinal lymphatic vessels are fewer in numbers, smaller in diameter, and lie deep in the mucosa 300–400 μm beneath the mucosal epithelium (Fig. 3) [7].

Blood vessels have open fenestrae with diameters ranging from 40 to 60 nm; however, they have properties similar to the continuous capillaries [8]. In contrast the lacteals are composed of endothelial cells that have no fenestrations. However, the junctions of these endothelial cells are widely separated and allow the passage of macromolecules such as chylomicra (75–600 nm diameter) into the lymphatic lumen. The lymphatic capillaries also have a fragmented basement membrane offering little barrier to the passage of solutes, fluids and large particles into lymphatic vessels [9]. The major pathway for the transport of fluids and particulate components from the interstitium into the lymphatic lumen is the intercellular route. Smaller particles ranging in diameter from 3 to 50 nm have been shown to pass through intermediate junctions (zonulae adhaerentes) of the endothelial cells

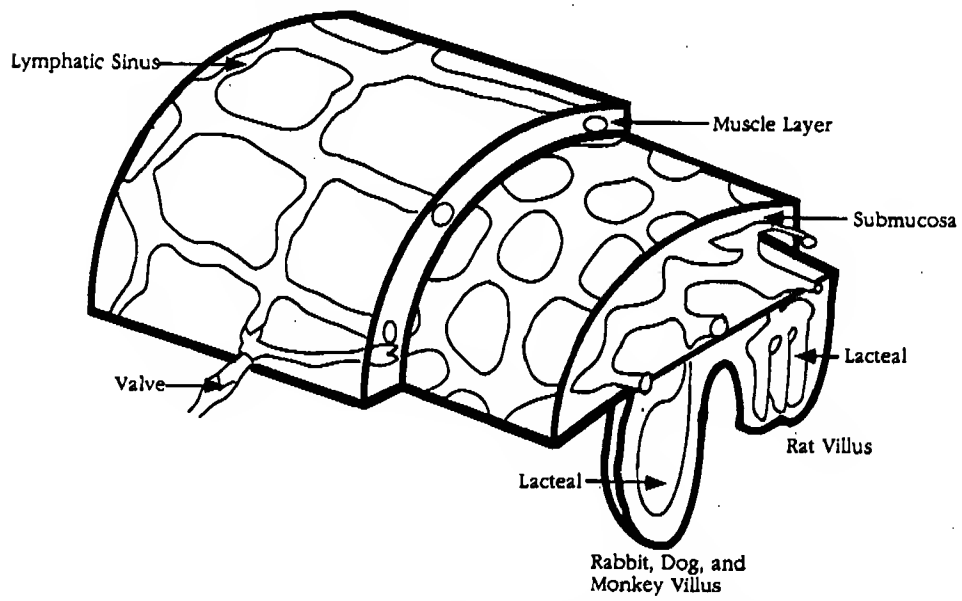

Fig. 1. Lymphatic network in small intestine. Mucosal, submucosal and muscle layer lymphatics are presented. As shown, lymphatics of the muscle layer are not directly connected with submucosal lymphatics, but both lymphatics merge into larger lymphatics near the mesenteric border (from Ref. 305).

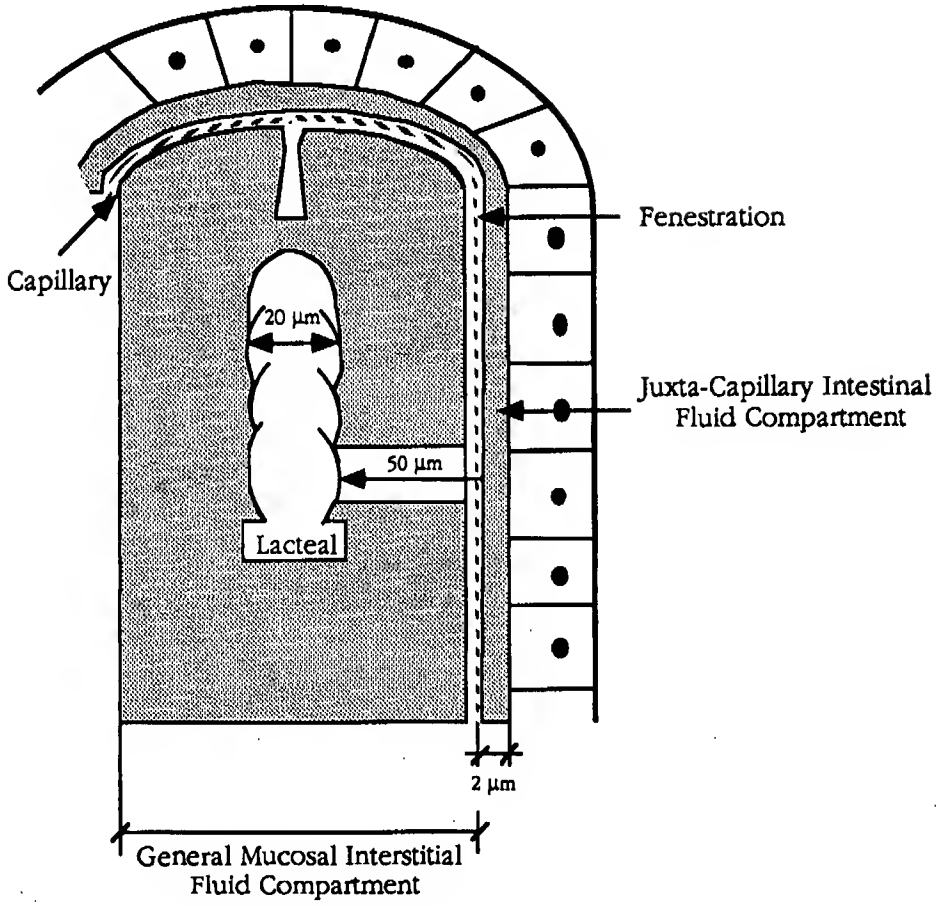

Fig. 2. Cross-section of an intestinal villus with blood and lymph microcirculation (from Ref. 4).

but not tight junctions (zonulae occludentes) [10]. Substances in this group can also be taken up by vesicles of approximately 50 nm diameter and transported across the cell by this route (Fig. 4).

Fluid from the lumen moves to the interstitium, which is composed mainly of collagen fibers and hyaluronic acid, a polymer of *N*-acetylglucosamine and glucuronic acid with a molecular mass ranging from a few thousand to several million daltons. Cross-linking between hyaluronic acid and collagen and other proteins creates a fine interstitial mesh, with a pore size of about 25 nm [11]. Since hyaluronic acid is anionic, the gel area has a net negative charge at physiologic pH and ionic strength [12]. When interstitial fluid volume rises during a meal from its normal 25 ml/100 g the porosity increases from 25 nm to approximately 100 nm, resulting in reduced frictional resistance to diffusion of macromolecules and an increased hydraulic conductivity [13]. This was demonstrated in a perfused cat ileum preparation where the authors studied the effects of net volume absorption rate on interstitial fluid volume, lymph flow and the excluded volume fraction for interstitial albumin. Their

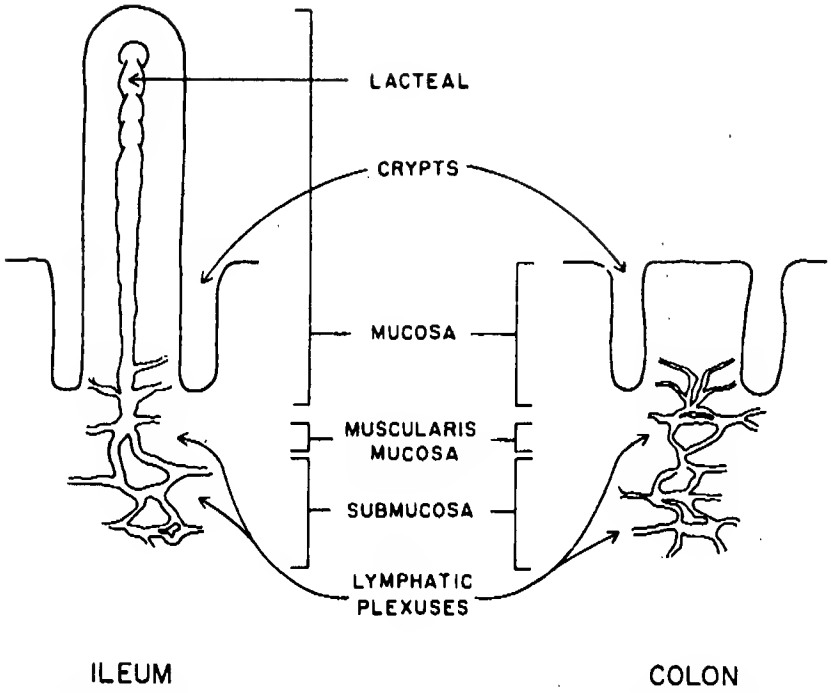

Fig. 3. Schematic representation of the mucosal-submucosal lymphatic microcirculation of the canine ileum and colon (from Ref. 7).

results indicate that fluid absorption increases the interstitial volume and lymph flow. Increased interstitial volume decreased the excluded volume fraction for albumin, suggesting that the degree of exclusion of a given molecule is inversely proportional to matrix hydration (Fig. 5).

The interstitial-to-lacteal hydrostatic pressure gradient is the major driving force for lymphatic filling and therefore determines lymph flow. Typically lymph flow is about 500–700 times less than blood flow [14]. When venous outflow pressures (P_v) were increased from 0 to 30 mmHg in an in situ cat intestinal loop, the interstitial fluid pressure increased from −1.8 to 5.3 mmHg. Lymph flow increased minimally over the lower venous outflow pressures (up to 10 mm), and there appeared to be a plateau at the higher venous outflow pressures (25 and 30 mmHg). However, a rapid change in lymph flow occurred in the range between 10 and 20 mmHg. The largest lymph flow observed was at P_v = 30 mmHg, creating approximately 7 times the lymph flow of 0 mmHg venous outflow pressure [15].

Several authors have reported an increased intestinal or thoracic duct lymph flow following a meal or fluid ingestion [3,16]. During net fluid absorption, the increase can be as high as 5–20 times the normal lymph flow when compared to the non-absorptive state [13]. However, the magnitude of increase appears to be quite variable and has been attributed to factors such as tonicity of the fluid ingested, portal vein pressure, intraenteric pressure, and GI motility. If these factors are held con-

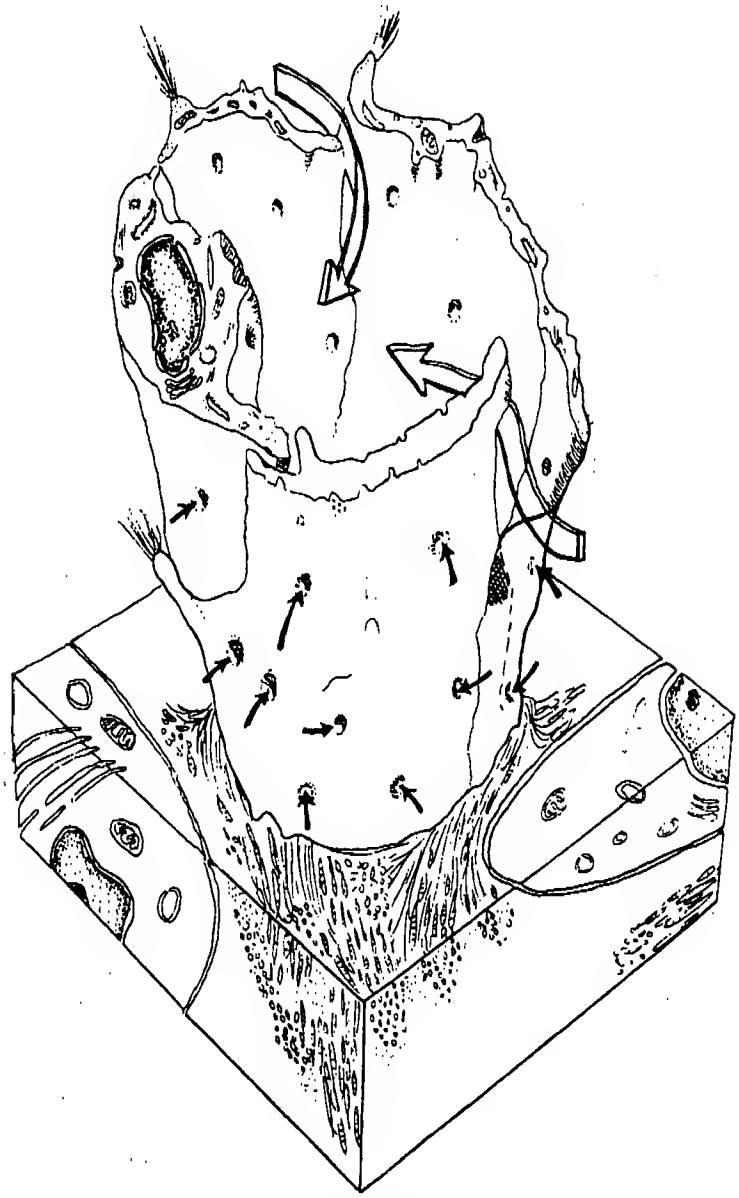

Fig. 4. Three-dimensional representation of a lacteal. The major passage way for the transport of fluids and particulate substrates from the interstitium into the lymph lumen is the intercellular cleft (long open arrows). The uptake of particulate components from the connective tissue front is also carried out by vesicles containing the particles. It is suggested that the movement of particles proceeds from the connective tissue front of the endothelium via vesicles (short filled arrows). The vesicles seem to merge or fuse with autophagic vacuoles (from Ref. 10).

stant or eliminated, the rate of fluid absorption becomes a major determinant of intestinal lymph flow. However, the overall lymphatic absorption rate in the

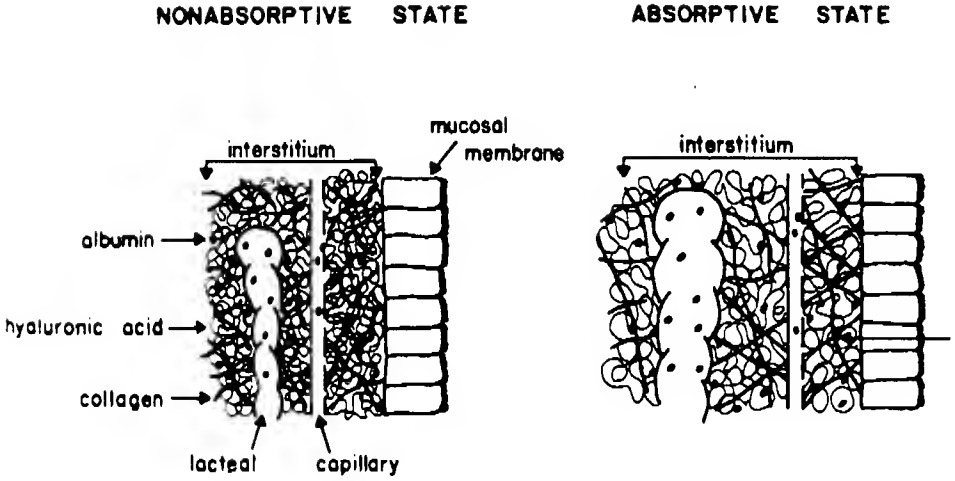

Fig. 5. Diagrammatic representation of intestinal interstitium in non-absorptive and absorptive states. In the nonabsorptive state albumin is distributed in only one fraction of the total matrix water and the lymph flow is low. During absorption, interstitial fluid volume and lymph flow increase and a greater portion of interstitial water becomes available for albumin to distribute in (from Ref. 13).

absorptive state does not exceed 15–20% of the total volume removed from the interstitium [17]. Glucose and electrolytes do not affect intestinal vascular permeability. However, there is a six- to sevenfold increase in intestinal lymph protein flux during glucose absorption [7,18].

Fat absorption produces an increase in lymphatic protein flux [3,19]. The reflection coefficient (σd) describes the fraction of the total osmotic pressure generated across a capillary membrane. Impermeable molecules yield 100% of their maximum osmotic pressure and $\sigma d = 1$, whereas freely permeable molecules do not generate an effective osmotic pressure and $\sigma d = 0$. Granger and coworkers [20] demonstrated that either cream or a mixture of bile and oleic acid produced a four- to sevenfold increase in lymphatic protein flux, and an increase in vascular permeability. σd decreased from a normal value of 0.92 to 0.68 with bile and oleic acid and to 0.71 with cream feeding [20]. Using the osmotic reflection coefficient values obtained for several endogenous proteins of varying molecular size (3.7–12 nm) the authors were able to calculate the pore size in the intestinal capillary wall. Minimal change was found for small pores during fat absorption (5.1 nm) compared to control (4.6 nm), however, large pores gained in size from 20 to 30 nm. Because the morphologic equivalent of the large pores in intestinal capillaries is considered to reside at the open fenestrae (approximately 20 nm radius), fat absorption must preferentially affect these structures [21]. Increased vascular permeability during fat absorption is due to the dramatic increase in plasma protein flux across the capillary wall. The equation, which relates convective and diffusive fluxes to the net flux of protein is:

$$J_{p,n} = J_{V,C}(1-\sigma d)C_p + PS\Delta C \quad (1)$$

where $J_{p,n}$ is the net transcapillary protein flux, $J_{V,C}$ is the steady-state transcapillary volume flow, σd is the reflection coefficient, C_p is the average protein concentration across the capillary wall, i.e. $(C_L + C_p)/2$, PS is the permeability surface area product and ΔC is the transcapillary protein concentration gradient $(C_L - C_p)$ [22]. This increased flux may be the result of an increase in perfused capillary surface or it may be due to an increase in capillary permeability [20]. Granger and coworkers [23] have demonstrated that plasma proteins move from blood to interstitium across filtering capillaries in the non-absorptive state (at normal portal pressure) by both convection (accounting for 90% of the total capillary flux) and diffusion. Under these conditions, the convective and diffusive processes are directionally coupled, in as much as both the flow of solvent and the concentration gradient are directed toward the interstitium. Convection is the major process and moves plasma proteins from blood to interstitium across filtering capillaries at a

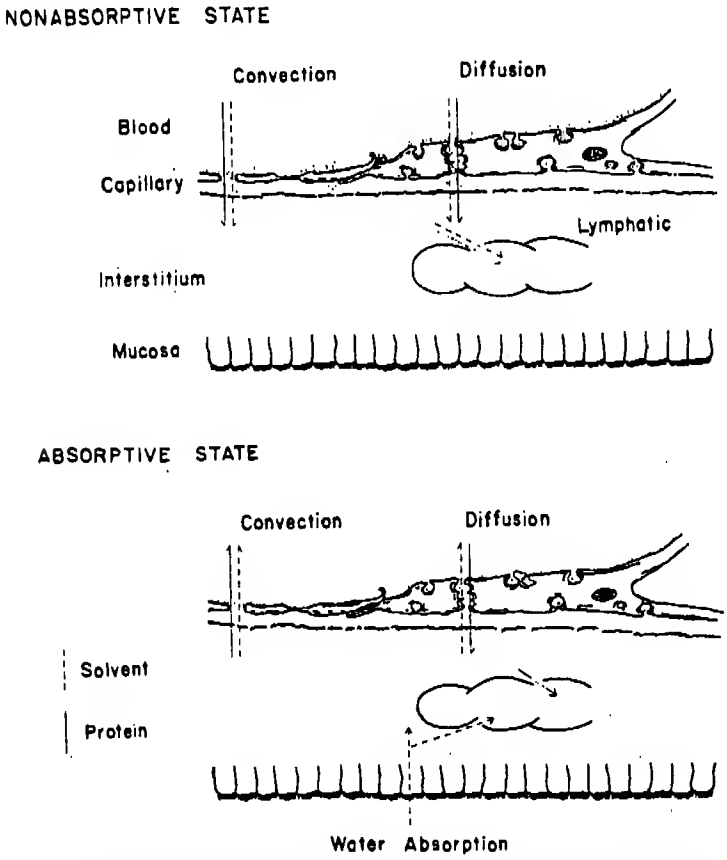

Fig. 6. Schematic representation of solvent and solute fluxes across capillary, lymphatic, and mucosal barriers in non-absorbing and absorbing small bowel (from Ref. 23).

normal portal pressure (non-absorbing intestine). In the absorptive state, mucosal interstitial fluid volume increases, which, in turn, causes mucosal interstitial fluid pressure to increase and tissue oncotic pressure to decrease. This opposes further capillary filtration and converts filtering to absorbing capillaries, and provides an increased driving force for lymphatic filling. Under these conditions, the flow of solvent across intestinal capillaries is directionally opposing the protein concentration gradient; therefore, the transcapillary convective flux of plasma protein is directionally opposite to the diffusive flux (Fig. 6). The result is a blood-tissue circulation of plasma proteins during absorption, where most of the proteins return to the circulation by convection across the walls of absorbing capillaries. The remainder return to lymph. The existence of a blood-tissue circulation of plasma proteins during absorption might be an important mechanism for the absorption of medium- and long-chain free fatty acids as well as drugs with a high protein binding capacity that are not incorporated into chylomicrons but enter the circulation in significant amounts bound to albumin [23].

Although most of the protein in the lymph is derived from the plasma by filtration, there are other sources. One source is the large number of plasma cells in the lamina propria of the intestinal mucosa. In dogs, mesenteric lymph contains considerable amounts of IgA arising from these cells [24]. The IgA concentration of the mesenteric lymph has been examined in a variety of animals [25]. For dogs, the ratio lymph/serum was 2.4, while the ratios in guinea pigs and rats were 4.5 and 13, respectively. It appears that the mesenteric lymphatics are the main route of newly synthesized IgA to the blood and it has been calculated that 90–97% of IgA in mesenteric lymph is synthesized in intestinal immunocytes.

Chylomicrons are formed in the enterocyte and leave the cell by exocytosis, passing through pores in the basement membrane of the epithelial cell. Chylomicrons traverse approximately 50 μm of interstitium to the lacteal, where they are taken up by a mechanism not yet fully understood [26–28]. In rats, Tso and coworkers [29] demonstrated that expansion of interstitial matrix resulting from water absorption as well as increased lymph flow after receiving an intravenous infusion of saline, enhanced the transport of chylomicrons from the enterocyte to the lacteal.

II.2. Absorption into lymph

There are two distinct tissues in the gut that allow access to the lymph vessels. The most wide-spread is absorptive tissue followed by lymphoid tissue composed of solitary and aggregated lymphoid follicles. This section discusses uptake mechanisms by absorptive tissue.

In rats, no preferential uptake into lymph was found for amino acids upon infusion of glycine and glycine oligopeptides into the duodenum [30,31]. Investigation of radioactive lymph protein after feeding [^{14}C]leucine indicated that amino acids absorbed in a mixture with cream enter the pool of amino acids in the mucosal cells and are incorporated into proteins and transported by the lymph. Support for this theory came from Jacobs and Largis [30]. These authors investigated the effect of protein synthesis inhibitors, such as puromycin and acetoxycycloheximide, on the content of labeled amino acids in newly synthesized proteins in the mesenteric

lymph of rats. Both compounds reduced the appearance of new proteins in the lymph, while the concentration of free amino acids rose.

Several studies also suggest that minute amounts of ingested proteins eventually reach the general circulation via lymph. Most of these studies use proteins which either provoke an immune response or confer passive immunization in the host after absorption. Alexander et al. [32] demonstrated lymphatic transport of egg white protein after gavage feeding to dogs.

Toxins of *Clostridium botulinum* are transported in the lymph [33]. The toxin exists in several types and molecular masses ranging from 9 to 900 kDa. Examination of the absorption behavior of type A (900 kDa) toxin in rats, rabbits and mice suggested that the upper small intestine was the major site of absorption. In rabbits more than half of the fraction absorbed was recovered in thoracic duct lymph.

Absorption studies with ^{3}H-labeled albumin have clearly indicated the existence of two independent routes of transport for absorbed proteins [34]. Approximately 2% of the administered amount of ^{3}H-labeled albumin was recovered intact over a period of 3 h. In mesenteric lymph and portal venous blood 0.76% and 1.11% were found, respectively. The integrity of the protein was verified by gel filtration and radioimmunoassay against monospecific rabbit anti-bovine serum albumin.

Among the intestinal proteolytic enzymes, elastase (24 kDa) appears to be absorbed in limited quantities. Studies with ^{131}I-labeled elastase in rats suggest that the lymphatic route may be important for transport [35]. The lymph concentration of immunoprecipitable radioactivity 7 h after delivery of 1 or 5 mg labeled elastase into the upper jejunum was 10–11 and 7–26 times greater than serum concentration. The total amount absorbed, however, was very small, 0.149% and 0.053% of the 1 mg and 5 mg dose, respectively. The authors calculated the dose absorbed via the lymphatics as 36% of the total absorbed elastase. Related studies in a rat everted sac experiment demonstrated that intact elastase is able to cross the intestinal wall to a limited degree [36]. The amount was between 0.46 to 0.63% for a 0.01% elastase solution, when determined by dialysis, electrophoresis, gel filtration, and immunoprecipitation. In vitro, the transport rate was higher (but not significantly) for a 0.1% elastase solution.

Pharmacokinetic studies of a glycoprotein complex (RU 41740) composed of two molecular weight fractions, 350 and 95 kDa, respectively, in different species (rats and monkeys) demonstrated a greater uptake in rats (7%) compared with monkeys (2%) [37]. Because of the size it can be assumed that the fraction absorbed enters the circulation via the lymphatics.

Yamashita and coworkers [38] have described a peptidomannan fraction designated as KS-2, a hot water extraction of mycelia of Basodiomycetes, with a molecular mass range from $6 \cdot 10^4$ to $9.5 \cdot 10^4$ Da which was absorbed partially via the portal vein (1.4% of the dose) and intestinal lymphatics (1.25% of the dose). The intestinal absorption of two enzymes, 1,2-[^{3}H]co-enzyme Q-10 [^{3}H]Q-10), and [^{131}I]lysozyme was investigated by Yuzuriha et al. [39]. 80% of the absorbed [^{3}H]Q-10 was recovered from the lymph; however, the portal vein was the main absorption pathway for [^{131}I]lysozyme. It was concluded that absorption into lymphatics was also dependent on the physicochemical properties of the macromolecules.

II.2(a). Persorption. Small amounts of particulate material may enter the host through the epithelium of the intestinal mucosa via the paracellular pathway by a phenomenon called persorption [40–45]. In several studies it was demonstrated that particulate matter such as diatoms, pollens, spores, cellulose particles, plant cells, starch granules, and others can cross the intestinal mucosa. The best results were obtained with hard particles in the range of 5–70 μm, although persorption will also occur for particles (poly(vinylchloride)) up to 150 μm. Whether or not persorbed particles are transported by the lymphatic system depends on their size [45]. Small particles were predominantly transported by the portal circulation and larger ones by lymph; however, these size ranges have not been clearly defined.

The persorption rate in humans was estimated at 1:50000 (±50%). This means 1 out of every 50000 particles will be absorbed, or, for example, approximately 2.5 mg out of 100 g cornstarch. Volkenheimer [44] also found that the persorption rate was dependent on the amount ingested and was age related, with younger subjects demonstrating a higher persorption rate; however, this difference was not quantitated. Drugs increasing GI motility, such as neostigmine or caffeine, were found to increase persorption, whereas the reverse is true for drugs, such as atropine and barbituric acid, that reduce motility [46]. Thus, mechanical factors were thought to be responsible. However, recently several authors found that cholinergic stimulation altered the macromolecular permeability of tight or occluding junctions of crypt epithelium [47,48]. From their studies it appears that reduced restriction in tight junctions due to the co-application of neostigmine or caffeine yielded higher persorption.

Persorption has also been described for *Candida albicans* in human volunteers and confirmed for *C. albicans* in dogs and primates [49,50]. 10^{12} *C. albicans* cells were delivered as a suspension to a volunteer. Blood and urine were collected for culture. The blood samples taken at 3 and 6 h each grew two Candida colonies. 31 colonies grew from the sediment of the urine taken after 2 h 45 min, and 8 from the one taken after 3 h 15 min. The rest of the blood and urine cultures remained negative up to 14 days. The authors assume that *C. albicans* causes systemic infection in two ways, the first by persorption and the second by penetrative growth.

Assuming that persorption is a particulate transport mechanism via the paracellular shunt, then one should also consider the underlying absorption phenomenon for nanospheres as persorption unless such nanospheres are taken up by phagocytosis in mucosal lymphoid tissue [50–51]. It has been shown that nanocapsules containing insulin are able to reduce plasma glucose levels over a prolonged period of time in diabetic rats when administered orally.

II.2(b) Targeting to the lipid pathway. Many of the recombinant substances that are orally administered are given in lipid vehicles and it seems reasonable to assume that they undergo co-absorption with lipids [56,57]. Alternatively, lipids may influence drug absorption indirectly, via physiologic mechanisms stimulated during lipid digestion [57–60]. Co-administration may be a tool to modulate lymph. For example, the relative order of the total lymph volume collected over a 24 h period following intragastric administration of several vehicles was: olive oil > ethanol

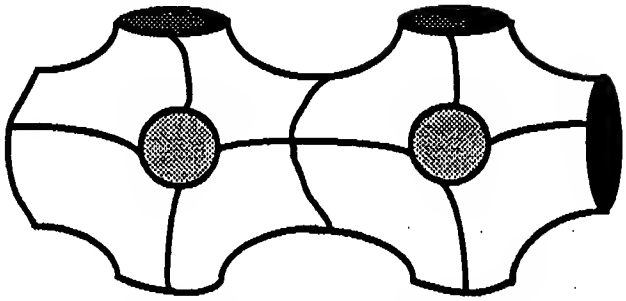

Fig. 7. Cubic monoglyceride structure based upon hexagon-shaped bilayer discs. The figure shows two polyhedra along one water channel (from Ref. 71).

10% and a commercial 10% safflower microemulsion (Liposyn 10%) > 2.5% sodium oleate > 1% sodium oleate > water [61]. These results, however, are not due to an increased number of chylomicrons or VLDL particles. Instead, during lipid absorption the enterocytes increase the size of the chylomicrons [62]. Egg PC increased lymphflow 5-fold and was absorbed almost evenly throughout the proximal and distal jejunum; furthermore, the main class of lipoproteins formed was VLDL with a particle diameter of 52 nm [63,64]. Thus the interaction of digestive enzymes with lipids, the formed lipidic phases, their absorption, and their modulation of physiology needs to be understood.

Orally ingested triglycerides undergo extensive hydrolysis in the lumen to yield the corresponding 2-monoglyceride and fatty acids, which are then solubilized in mixed micelles. However, the implication that there are only oil and micellar phases during fat digestion might be an oversimplification [65]. Fat digestion results in the formation of at least four co-existing phases: namely, the oil phase, the micellar phase, the calcium soap phase (lamellar liquid crystalline phase) and the viscous isotropic phase (cubic liquid crystalline phase) [66,67]. Under physiologic conditions, however, extensive calcium soap formation would not be expected because monoglycerides inhibit the soap formation by acting as molecular spacers preventing calcium from binding to the fatty acids [66]. The viscous isotropic phase forms subsequently to the cessation of calcium soap formation [65]. Bile salts are apparently not a required part of this phase because it can form in their absence [66,68]. The surface tension of the isotropic phase is low enough so that it can take any shape in water. The structure may be similar to the structure of the cubic phases of lipid-water systems and is unique regarding their media, one lipid and one water, both continuous throughout [69,70] (Fig. 7). Although the necessity of the cubic phase in the mucosal fat absorption has not yet been proven definitively, there is evidence that it may be so.

There is vast literature regarding the formation of a cubic liquid crystalline phase upon interaction of lipids with water [70–79]. For example, the fusogenic glycerolmonooleate, alone or as mixtures with phospholipids, forms a bicontinuous phase over a wide lipid/water ratio [72,76]. The dimension of the water channels is 5 nm in diameter [68]. It is conceivable that such a structure is able to incorporate large hydrophilic compounds. Moreover, it has been demonstrated that proteins with a

wide variation in size (14–150 kDa) are able to induce a cubic protein-monoolein-water phase [56,80]. Formulation of a peptide/protein in such a cubic liquid crystalline phase could target the compound to the lymphatics.

Utilization of the triglyceride pathway for targeting macromolecules to the lymphatic vessels is well documented [81–88]. In order to transfer CsA selectively into the thoracic lymphatic system, Yanagawa et al. [85] administered CsA to rats at a dose of 10 mg/kg orally in lipid microspheres composed of olive and soybean oil, egg PC, and glycerol, with an average particle size of 191 nm. Intestinal digestion of the lipids yields products capable of undergoing a phase transition into a cubic liquid crystalline phase and thereby delivering the drug to the lymphatic vessels. After two hours, CsA thoracic lymph concentration, when administered as lipid microspheres, was approximately 46 times greater than after dosing with a conventional preparation (Fig. 8). For the lipid microspheres preparation, maximal lymph levels were achieved within 2 h (3608.6 ng/ml), while the conventional formulation levels were still increasing, but quantitatively much lower.

Cho and Flynn [86] have introduced a water-in-oil microemulsion containing bovine insulin. The lipid components roughly equaled their proportion within chylomicrons. The proportions of cholesterol, PC, and an esterified fatty acid were selected as, respectively, 1:2:14. In order to protect insulin from degradation by proteolytic enzymes, an undisclosed amount of the protease inhibitor aprotinin was added. The formulation was designed so that a significant fraction of the administered drug was targeted to the liver by co-transport with chylomicrons and chylomicron remnants. Three patients, all with a long history of diabetes, received a dose of 1 IU/kg as a liquid. All three patients demonstrated a significant reduction in blood glucose (Fig. 9). However, the data presented are very limited and bioavailability or total duration of blood glucose reduction are not reported. According to their patent [87], a similar formulation composed of an oil phase (oleic acid, egg PC, and cholesterol) and a mixture of surfactants (glycerol monooleate and polysorbate 80) coated onto a solid core carrier significantly lowered the clinical blood sugar level. The formulation was administered together with 250 ml of water. Upon dilution of 3–5 times in the duodenum [66] the ratio of the mixture surfactant-lipid to water is between 20/80 and 40/60. These ratios fall well into the range of the cubical phase of glycerol monooleate/phospholipid mixtures [72], which have the ability to promote membrane fusion [56]. Since there is currently no published experimental evidence to confirm or deny that such a lipid-water phase may transport an incorporated peptide/protein into an enterocyte, one can only speculate about the uptake mechanism. However, it is known that there is a close relationship between the rate of absorption and the percentage of lymph chylomicrons, the integration of fatty acids into lymph triglycerides, and the similarity between the lymphatic triglyceride fatty acid composition and that of the test infusate [89–92]. Feeding high amounts of oleic acid, but not its isomer elaidic acid, turns the chylomicron production on and thereby increases the recovery of the fatty acid in chylomicrons [93,94]. The authors explain their results as a detouring of fatty acids from the portal route to the lymphatic system, by simply saturating the former. It also seems that the isomers, oleic and elaidic acid, are processed differ-

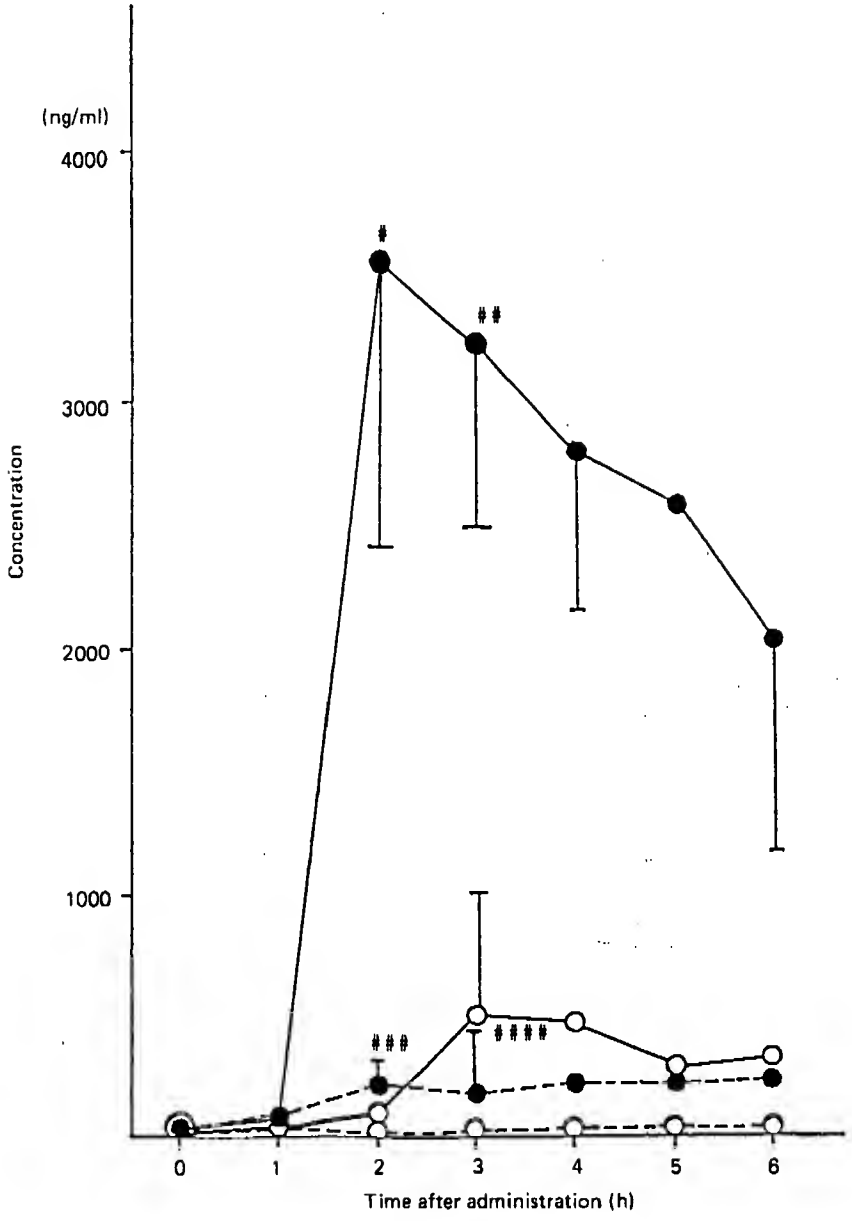

Fig. 8. Cyclosporin A concentrations in lymph and blood against time. CsA lymph concentration after administration in lipid microspheres (●—●); CsA lymph concentration after administration in a conventional formulation (○—○); CsA plasma concentration after administration in lipid microspheres (●------●); CsA plasma concentration after administration in a conventional formulation (○------○). Levels of significance: lipid microspheres vs. conventional formulation in lymph (# P <0.1; ## P<0.05); lipid microspheres vs. conventional formulation in plasma (###, P<0.02; ####, P<0.05) (from Ref. 85).

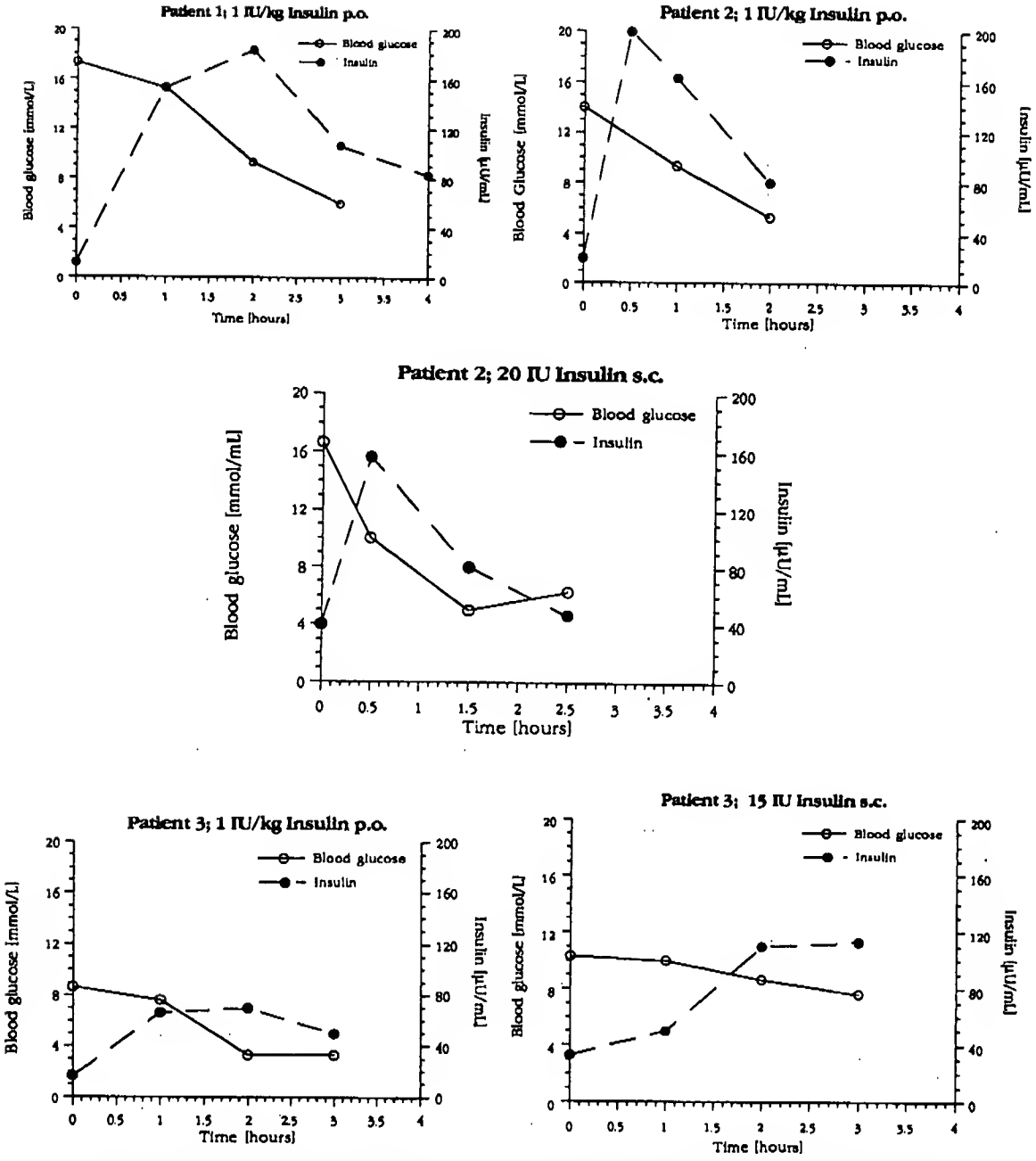

Fig. 9. Serum insulin and blood glucose levels in three patients with insulin-dependent diabetes mellitus after a single dose of oral and subcutaneous insulin (from Ref. 86). Left vertical axes: blood glucose (mmol/l); right vertical axes: insulin (µU/ml); horizontal axes: time (h).

ently by the enterocytes [94]. In similar work Cheema et al. [59] found that the longer the chain length and the greater the degree of unsaturation of the fatty acids, the faster the onset of chylomicron synthesis. Their results suggest that linoleic acid is the most suitable of the lipid vehicles investigated for the delivery of drugs into the lymphatic system, since it rapidly stimulated high levels of chylomicrons in the lymph. Conversely, long chain and saturated fatty acids, such as palmitic acid, caused a marked increase in VLDL transport [94,95]. Since the main lipid in Cho and Flynn's formulation is oleic acid [87], it is conceivable that in addition to the suggested vehicle effect a modulation of the physiology takes place, thereby increasing protein uptake via the triglyceride pathway.

Similarly, another patent discloses an emulsion system for oral delivery including peptides [96]. An LH-agonist was administered in a formulation composed of an oil phase (olive oil, oleic acid, peppermint oil), surfactant (Emulphor®), water and *N*-methyl-2-pyrrolidone. The oil phase is represented by insoluble, non-swelling amphiphilic lipids which account for over 40% of the formulation. The water content in this composition is only 4%. Since interaction of different lipid classes with water can cause cubic liquid crystalline phases to be formed [97], it is likely that such a phase is formed resulting in a bioavailability of up to 85% for leuprolide following intraduodenal administration in rats. Additionally, modulation of chylomicron synthesis could be partially responsible for this excellent result.

A similar study was conducted by Liedtke et al. in mice [88]. Although the exact details of their formulation are not given, it was described as a liquid lipid emulsion at 38°C composed of acylglycerides and a solution of human insulin. The maximal decrease in blood glucose was achieved 2 h following the oral administration of the lipid formulation and the bioavailability after s.c. and p.o. administration was comparable. It is believed that the underlying uptake mechanism is similar to the one described above.

Ritschel et al. [98–100] have demonstrated that microemulsions similar to those above can increase the oral bioavailability of peptides in various species. These W/O microemulsions have a low water content, sometimes as low as 8% [98]. From their data, it is also obvious that parameters other than droplet size in the microemulsions are critical for absorption. For example, branched fatty acids in the oil phase lowered the CsA uptake compared to unbranched molecules. The microemulsion (ME–15) used for insulin was composed of an oil phase (esterified fatty acid), a mixture of surfactant and co-surfactant (polyoxylated caprylcaprinic acid glycerides and polyglycerol isostearate) and water [100]. The bioavailability in beagle dogs, following administration of encapsulated ME–15, was 5%. Pinocytotic uptake of fat particles can be ruled out almost with certainty after fat absorption studies showed no evidence for such an entry mechanism [101,102]. Again, it is conceivable that this formulation once in the duodenum forms a cubic liquid crystalline phase upon interaction with the pancreatic juice.

In a similar study Takada et al. [103] administered solid dispersions to rats composed of CsA, at a dose of 7 mg/kg, surfactant (polyoxyethylated, hydrogenated castor oil, HCO–60) with selective lymphatic transporting capability, and an enteric coating material. Their procedure for preparation was the following: CsA,

HCO–60 and an enteric coating polymer such as cellulose acetate phthalate (CAP), were dissolved in methanol. The solution was stirred in a mortar until granules resulted. These granules were dried under vacuum overnight at room temperature. The dried product was pulverized and screened through a 50 mesh screen. The preparation was placed into tubes which were inserted into the rat gut via an incision and 0.5 ml of 0.1 N HCl was injected. The highest achieved lymph level (7680 ng/ml) occurred after administration of a solid dispersion of CsA in hydroxypropyl methylcellulose phthalate (HP-55®)). The percentage transferred into the lymphatics after 6 h was approximately 2% of the administered dose.

II.2(c) Physicochemical properties for lymphatic uptake and possible chylomicron incorporation. The ability of a compound to gain access to the lymphatic vessels depends upon, among other things, its molecular size [104]. Blood vessels have fenestrae with diameters between 40 and 60 nm, whereas lymph vessels possess junctions that are widely separated and therefore allow the passage of chylomicrons (up to 600 nm in diameter) into the lymphatic lumen. Smaller substances can be forced into the lymph vessels by increasing their size via complexation to a large molecular weight carrier [105]. Small molecules gain access to the lymphatic lumen by incorporation into chylomicrons, depending upon their hydrophobicity and lipid solubility [106].

The permeselectivity of high molecular weight dextrans absorbed from the small intestine to the lymphatic vessels in comparison to the blood capillary system was demonstrated using in situ rat closed loop experiments [104]. These authors found that the levels for dextrans with molecular mass greater than 40 kDa were significantly higher in the lymph than in plasma. They concluded that the threshold molecular weight for a change in mechanism of absorption from the intestinal lumen, which results in higher lymph than plasma levels, was between 17.5 kDa and 39 kDa. In similar studies Muranishi and coworkers [107] observed a selective lymphatic uptake for dextrans with a molecular mass which was higher than about 20 kDa following administration in mixed micelles in the large intestine.

Yoshikawa and coworkers [105] have described a bifunctional delivery system for the selective transfer of small and poorly absorbed drugs into the lymphatics via the enteral route. Bleomycin (BLM), a cationic glycopeptide (molecular mass 1.5 kDa), was administered as a BLM-dextran sulfate complex using mixed micelles as an absorption promoter. The authors proposed the following mechanism for lymphatic transfer after administration into the lumen of the small intestine. Approximately half of the complex remains intact in the lumen, evidenced by evaluating the luminal solution using gel filtration and the determination of the different fractions for BLM and dextran. Complexed and free BLM enter the epithelial barrier only when aided by the mixed micelles as an absorption promoter. In the tissue more BLM dissociates from the complex reducing the complexed drug to about 15%. The free bleomycin then transfers almost equally into blood and lymph, while the complexed drug preferentially enters the lymph (Fig. 10). Therefore, the effectiveness of the selective transfer of the system into the lymph depends on its stability in the tissue. The authors also investigated large intestinal tissue for remaining

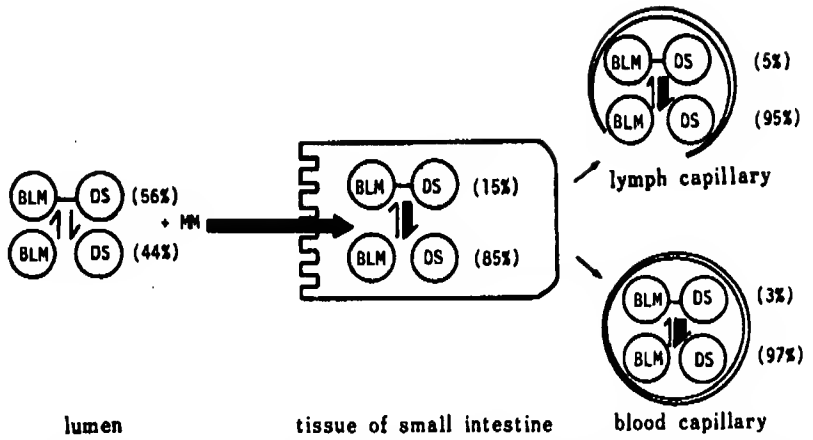

Fig. 10. Proposed mechanism of lymphatic transfer of bleomycin from lumen of small intestine by administration of bifunctional delivery system (from Ref. 105).

BLM-complex. Since 44% remained intact in the large intestinal tissue compared to 15% in the small intestinal tissue, this bifunctional delivery system seems well suited for colonic lymphatic delivery.

In a similar approach Kaji and coworker [108] used a β-cyclodextrin polymer as a macromolecular carrier for 1-hexylcarbamoyl-5-fluorouracil (HCFU). Delivery of the carrier system in a micellar solution (bifunctional system) into the large intestine of rats selectively increased the transfer into lymph. Concentrations of HCFU in lymph relative to plasma (L/P) peaked after 2 h, and the peak height was about 25 times that in plasma. In contrast, application of the delivery system as a buffered solution yielded a plateau after 1.5 h and the measured concentration was about 10 times that in plasma. Thus, mixed micelles composed of linoleic acid (2%) and polyoxyethylene lauryl ether (4%) in distilled water, enhanced absorption of the delivery system into the lymph system.

To date, partition coefficient (P_c), which is the ratio of the equilibrium partitioning of a compound between octanol and an aqueous phase, is most commonly used to describe lipophilicity. Very polar compounds ($\log P_c < 0$) transport slowly across the cell membrane due to poor membrane partitioning properties. These compounds will cross the epithelium primarily via the paracellular pathway, restricted by the presence of tight junctions. As the partitioning of the compound increases, membrane interaction increases. At some point, the permeability will be maximized, $\log P_c$ values of about 1–3 are presumedly optimal [109–114]. At higher $\log P_c$ values the unstirred water layer becomes the rate-determining barrier for transport to the epithelium [115,116].

Lipophilicity, however, does not take into account lipid solubility. Charman et al. [117] stressed the importance of lipid solubility for lymphatic transport. Hexachlorobenzene (HBC) and 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) were chosen as two model compounds with similar octanol/water partition coefficients ($\log P_{cDDT} = 6.19$; $\log P_{cHBC} = 6.53$), however, their lipid solubility in peanut oil differs by more than one order of magnitude (97.5 mg DDT/ml lipid; 7.5 mg

HBC/ml lipid). Cumulative lymphatic transport (0–10 h) was 33.5% for DDT, and 2.3% for HBC, respectively. Similarly, Pocock [106] used rats to demonstrate that cumulative DDT uptake (12 h) into thoracic lymph was 60% of the dose, and 97% of the amount found in the lymph was incorporated into the triglyceride core of chylomicra. Conversely, penclomedine has a log P_c of 5.48, an order of magnitude below that for DDT or HBC, and a lipid solubility of 175 mg/ml, which in turn is much greater than the solubility for DDT, but cumulative lymph transport of penclomedine (% dose) was only 2.0% [118]. The lower cumulative lymphatic transport of penclomedine highlights the importance of the partition coefficient on lymphatic uptake.

Cyclosporin A, an immunosuppressive drug whose physiologic target is the lymphatic microenvironment rather than the blood, targets to T helper cells and other lymphocytes. The lipophilicity of the compound had been considered to be the prime factor governing lymphatic uptake. Structurally, CsA is a cyclic undecapeptide with a molecular mass of 1202 Da and an octanol/Ringer's buffer partition coefficient of log P_c = 2.99 [119,120]. However, in rats Ueda et al. [119] found that only 2% (0.5% of the given dose) of the total amount absorbed drug was lymphatically transported following administration in a formulation believed to target the triglyceride pathway (absolute ethanol, Labrafil®-M 1944CS, and olive oil (18:42:40)). The authors attribute their results to poor permeability of the compound, and/or hindered or prevented incorporation into the core of chylomicrons because of the bulkiness of CsA. Alternatively one can speculate that the log P_c (2.99) might be too low for chylomicron incorporation (for references see Charman and Stella in this issue [306]).

In contrast several authors demonstrated high lymph levels following intragastric (i.g.) and intraduodenal (i.d.) administration of CsA and human fibroblast interferon (HuIFN-β) as mixed micelles composed of linolic acid (5.0 w/v%) and HCO–60 (8.0 w/v%) [121–124]. These results can be attributed to an enhancing effect of the fusogenic lipid formulation, which in this particular case exceeds the importance of the equilibrium partitioning of a compound between octanol and an aqueous phase.

II.2(d) Endocytosis. Absorption cannot be discussed without a brief review of endocytotic processes (for literature see Refs. 125–129). Endocytosis is a membranal process of transporting extracellular material to the interior of the cell [130]. Generally two types of endocytosis are distinguished: phagocytosis and pinocytosis [128]. Phagocytosis is associated with the internalization of particles greater than 0.5 μm and occurs in most cells. The term pinocytosis refers to both fluid phase pinocytosis (uptake of extracellular fluid and dissolved solutes) and adsorptive endocytosis (macromolecules bound to the membrane). Pinocytosis involves the formation of vesicles between 100 and 200 nm in diameter. Following the formation, vesicles can have distinct pathways within cells (Fig. 11) [129]. Most vesicles fuse with preexisting, membrane-bound granules called lysosomes. In pinocytosis the total amount of cell-associated marker is 1% or less of the administered load vs. 50% or more in typical phagocytic systems [129]. Macromolecules are rarely

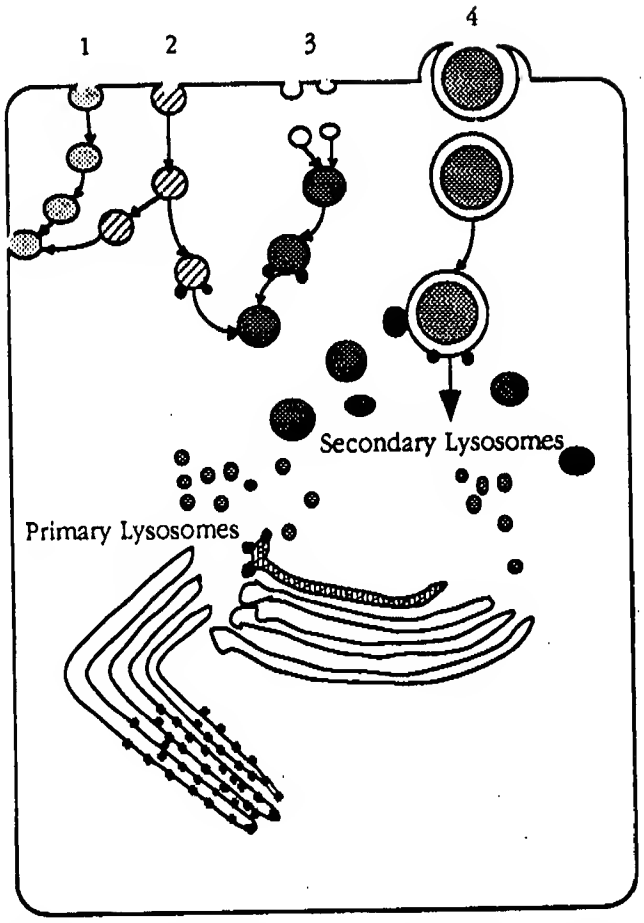

Fig. 11. Illustration of various endocytotic pathways. 1, fluid-phase pinocytic vesicle, which traverses the cytoplasm without fusing with lysosomes and discharges its contents on another surface of the cell. 2, adsorptive pinocytosis in which solute is initially bound to the cell surface through specific or non-specific determinants. After interiorization the vesicle moves through the cytoplasm and fuses either with the plasma membrane as in step 1, or with primary lysosomes or secondary lysosomes. 3, fluid-phase in which the vesicles fuse with each other to form larger structures and subsequently with lysosomes to form a new population of secondary lysosomes or add substrate to existing secondary lysosomes. 4, phagocytosis, in which a solid particle is interiorized, excluding fluid-phase constituents of the external milieu. The phagosome encloses the particle and usually fuses with primary and/or secondary lysosomes to form a phagolysosome (from Ref. 129).

expelled intact from the lysosomes. Occasionally endocytic vacuoles manage to escape fusion with lysosomes and traverse the cell. This phenomenon is referred to as transcytosis and is observed especially in the M cells of PP [131–133]. Macromolecular uptake, upon binding to a specific receptor, is referred to as receptor-mediated endocytosis. The uptake of receptor-bound molecules and small collections of extracellular fluid is mediated by coated pits. For each endocytotic process a variety of marker compounds exists (Table I).

TABLE I

MARKER COMPOUNDS TO INVESTIGATE DIFFERENT ENDOCYTOTIC PROCESSES
From. Refs. 128 and 129.

Type of endocytosis	Compound(s)
Adsorptive	gamma globulin, lectin(s), polyanions (heparin)
Fluid phase	colloid gold, dextran, ferritin, HRP, inulin, PVP
Phagocytosis	latex, paraffin oil emulsions, starch granules
Receptor mediated	bacterial toxins, viruses (reovirus, poliovirus)

To the authors' knowledge there is limited literature about endocytosis in non-lymphoid tissue [134–138]. Sanders and Ashworth [134] found that latex particles (200 nm) were taken up into jejunal absorptive cells of adult rats, and observed in intact intracellular vesicles. These vesicles were released into the interstitium where they migrated into both lymphatic lacteals and blood capillaries. After intraluminal injection of HRP into rat-ligated segments, Cornell et al. [135] found the fluid-phase marker within membrane-limited cytoplasmic canalicular, vesicular, and vacuolar structures. The sequestration of the compound within membrane-limited structures suggests a specific excretion mechanism such as exocytosis. After infusion of 30 mg per kg of the enzyme into the jejunum of rats, Warshaw et al. [138] were able to demonstrate the presence of HRP in venous blood and lymph. In this study, however, it was not possible to determine the amount absorbed by either route. Cytochemical studies led to the assumption that most of the absorbed enzyme was transported across the mucosa by pinocytosis rather than by passive diffusion through the epithelium, or passage through intercellular junctions. Heyman et al. [139] measured the flux of peroxidase in adult rabbit jejunum. 97% of the dose was degraded while crossing the membrane, suggesting passage through lysosomes after endocytosis. Vesicle-mediated transcellular movement of peroxidase was observed in both stratified and columnar epithelia of fetal rat small intestine and colon [140].

In an attempt to demonstrate lymphatic absorption, Tsujii et al. [141] performed a perfusion experiment using an anti-elastase rabbit antibody, and showed that elastase can be absorbed without decomposition through healthy rat jejunum epithelial cells by a pinocytotic process and transported into the blood and lymph.

Woodley and Naisbett [136] selected tomato lectin as a test compound. This lectin is less susceptible to digestion in the brush border membranes of the gastrointestinal tract than bovine serum albumin (BSA). In an in vivo investigation the authors assessed the IgG response following oral and i.p. administration of the lectin. The response after oral feeding was 4 times greater than that for i.p. administration. These immunization experiments, however, don't quantify exactly what % of the dose enters the system in an immunologically active form. Nevertheless, the authors were able to demonstrate a response with an amount as low as 10 μg.

Immunoglobulins bind selectively to receptors and subsequently are transported from the intestinal lumen into the circulation [142]. Rodewald found that IgG-receptors are located within the duodenum and proximal jejunum in rats. In these

areas, receptors are limited to absorptive cells that line the upper portion of individual villis [143]. Interestingly, Hasegawa and co-workers [144] observed that IgG avoids degradation in the proximal intestinal cells because vesicles in which it is transported do not fuse with lysosomes, while IgG in the distal small intestine is degraded in lysosomal organelles. Use of such a transport mechanism for transcytosis of macromolecules seems attractive. In fact, IgG-coated liposomes carried poly(vinylpyrrolidone) and inulin across the epithelium in fetal rabbit and suckling rats much more efficiently than uncoated liposomes. A similar result was observed with insulin entrapped in IgG-coated vesicles. Although lymphatic absorption was not attempted, one can safely assume by taking the size of liposomes into account that they primarily gain access to lymph. This approach, however, is restricted to very young animals, since, in adults, Fc receptors are no longer expressed [145].

More emphasis must be directed to investigations that lead to a better understanding of the molecular mechanisms responsible for cell sorting. To date, only class IV-receptor-ligand complexes are known to 'transcytose' across polarized cells, thereby delivering the ligand to the opposite side of the cell [127].

II.2(e) Site-specific delivery. Site-specific application by administration into the rectum has also been shown to be suitable for lymph targeting. Yoshikawa and Muranishi [146] were able to selectively deliver cytokines, such as interferon (IFN) and tumor necrosis factor (TNF) into the lymphatics using mixed micelles (MM) composed of fusogenic lipids. The absorbed IFN was selectively delivered into the lymph from the colorectum of the rat, whereas the same formulation yielded similar levels in lymph and blood after oral administration. Yoshikawa et al. [147] were able to selectively deliver IFN into lymph by using suppositories containing linoleic acid and HCO–60, resulting in little IFN detected in serum. The bioavailability was about 25% of that obtained from the rectal administration of an MM solution composed of HCO–60 and linoleic acid [148]. The authors explain the lower bioavailability from suppositories when compared with an MM solution as a result of the limited dissolution of drug and adjuvants when given as a suppository. Furthermore, the diffusion rate in the molten suppository may be lower than in the MM solution.

Nishihata et al. [149] investigated the effect of adjuvants on the lymphatic uptake of pepleomycin sulfate after rectal administration to rats. 5-Methoxysalicylate increased lymphatic transport, whereas the sodium salt of phenylalaninamide of ethylacetoacetate and diclofenac did not. The authors believe that the enhancing effect of 5-methoxysalicylate might be due to the suppression of the vascular permeability for pepleomycin sulfate. This finding suggests that adjuvants may have an impact on the vascular permeability as well as mucosal membrane permeability.

In another study, the transport of oral CsA, from the gastrointestinal tract in rats, into both the systemic circulation and the thoracic lymph was investigated with respect to the effects of biologic factors, such as presence of bile, absorption site, and the type of formulation, such as polyoxylated hydrogenated castor oil (HCO–60), propylene glycol (PG), and poly(ethylene glycol) (PEG 400). There was no great variation in terms of systemic availability among the different absorption sites

including the proximal segment of the upper small intestine (15 cm distal to the insertion of the bile duct), the segment between the bile duct and ileocecal valve (15 cm), and the small bowel proximal to the ileocecal valve (30 cm). Lymphatic availability, however, showed great differences with the lowest lymph absorption occurring from the distal small intestine. From their results the authors concluded that for increasing systemic availability, absorption site is of minor importance; however, solvents such as HCO–60 or PG are essential. In contrast, optimal lymphatic bioavailability was only achieved from the proximal small intestinal segment. Bile was essential in both lymphatic and systemic bioavailability. Earlier studies from Garvey and coworkers [150] confirm these findings with a 10-fold decrease of CsA lymph concentration in pancreatectomized dogs. Similar results were achieved by Ritschel [100] who compared mean CsA blood levels vs. time following small intestine administration to control and bile duct-ligated rats. The plasma peak was 3 times greater in the control group compared to ligated rats. Following insulin application into the small intestine of rats blood glucose level fell 32% below the base level. But there was no reduction beyond base level upon applications into stomach, large intestine, and rectum.

II.2(f) Conclusion regarding absorption via non-lymphoid tissue. The GI tract allows macromolecules such as peptides or proteins to enter the lymphatic system to a limited extent. However, in order to target the lymphatics efficiently, one has to consider: (a) the molecular size of the drug itself or its carrier system, which should be greater than 20 kDa; smaller compounds (e.g., peptides) can be complexed with a macromolecular carrier in order to achieve the desired size and then administered in the presence of absorption promoters (bifunctional systems); (b) the vehicle effect, where the most promising vehicles seem to consist of a mixture of lipid and surfactant capable of directing the compound to chylomicrons and are capable of undergoing a phase transition into a cubic liquid crystalline phase; (c) the physicochemical properties of the compound should allow high partitioning into the chylomicron, while solubility in peanut oil can be used as an estimate of potential lymph partitioning. Endocytosis with respect to oral lymph targeting via non-lymphoid tissue is apparently not of great importance.

III. Absorption into lymphatic vessels via lymphoid tissue

For macromolecules, the potential to traverse the luminal and basal membranes in the GI tract is very limited. One possible pathway is passive transport through aqueous pores; however, this is generally restricted to water-soluble molecules of relatively small ($M_r < 1000$) molecular size. On the other hand, most mammalian cells, including those of the intestinal tract, have some capacity to endocytose macromolecules. Among the different intestinal components, PP are known to retain their endocytotic capabilities from birth to death. Their function is 'antigen sampling'; thus, they belong to the immunologic system [151]. Solitary lymphatic follicles are considered to be structural and functional equivalents to PP [152–155]. Targeting to these specialized tissues might be beneficial for a variety of com-

pounds, such as lymphokines and immunomodulatory substances.

III.1. Physiology of Peyer's patches

In 1676 Peyer described patches belonging to the gut-associated lymphoid tissue (GALT) consisting of aggregated lymphoid follicles [156]. As much as 25% of the GI mucosa is GALT [157] The shape of PP is generally rectangular or oval, and they are usually situated on the antimesenteric border of the intestine. PP extend through the luminal epithelium, lamina propria, and lamina submucosa. Lymphatic vessels are located under the epithelial layer adjacent to the external muscle layer. Post-capillary venules are located in internodular zones (Fig. 12) [158,159]. Patches are only one follicle thick, the follicle being intimately related to the overlying epithelium [160]. The structural elements of a PP are: (a) germinal center; (b) follicular area; (c) dome area; and (d) parafollicular area [161]. Quantitatively, the germinal center, follicular area, and parafollicular area are similar in volume, about 30% each and the dome area constitutes only a small portion of about 10% [162]. The epithelium over the dome area differs from elsewhere in the GI tract, since it is composed of cuboidal rather than columnar cells.

The number and location of PP varies widely between species and individuals (Table II) and is also age dependent. In humans and adult rats, PP are more numerous in the distal ileum, whereas in mice they are more uniformly distributed over the small intestine. However, in 8-week-old pigs, there are twice as many PP in the duodenum than in any other region [163]. In humans their number increases gradually through the end of puberty and thereafter a gradual and fairly equal reduction in the number of patches is found. A similar trend was also reported for

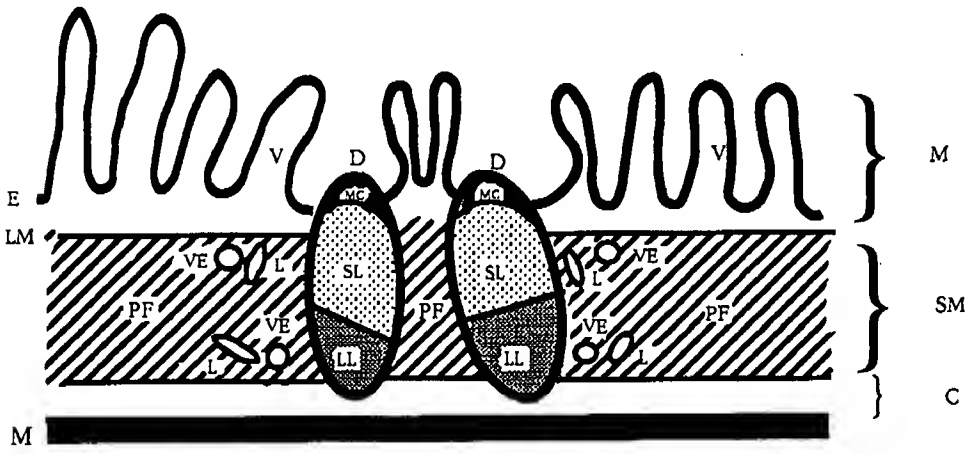

Fig. 12. Schematic diagram of the Peyer's patch showing the topographic areas. The intestinal lumen is in the upper and the serosal site is in the lower part of the diagram. *C*, connective tissue; *D*, dome area; *E*, epithelia; *L*, lymph vessel; *LL*, large-lymphocyte zone; *LM*, lamina muscularis mucosae; *M*, mucosa; *MC*, mixed cell zone; *PF*, parafollicular area; *SL*, small-lymphocyte zone; *SM*, submucosa; *V*, villi; *VE*, vein.

TABLE II

NUMBER OF PEYER'S PATCHES IN DIFFERENT SPECIES AND IN RELATION TO AGE
n.d., not disclosed

Species	Age	Number PP		Ref.
		range	mean	
Bovine	1.5–10 yrs	22–45	n.d.	164
	10–14 yrs	14–36	n.d.	164
Canine	0.5–1 yr	26–29	n.d.	302
Chicken	10 days	1–2	n.d.	225
	4 mths	5	n.d.	225
Horse	new born	245–320	n.d.	164
Man	1–3 yrs	122–244	161	160
	12–14 yrs	122–305	239	160
	15–19 yrs	170–316	220	160
	21–29 yrs	109–201	161	160
	32–50 yrs	104–217	134	160
	51–95 yrs	59–169	123	160
Mice	n.d.	6–10	n.d.	160
dd-mice	50–60 days	6–12	9	158
C57 Blk-mice	1 mth–1.5 yrs	4–12	6	303
C-mice	1 mth–1.5 yrs	4–13	8	303
Swiss-mice	1 mth–1.5 yrs	4–12	8	303
dba-mice	1 mth–1.5 yrs	5–14	9	303
C3H-mice	1 mth–1.5 yrs	6–14	11	303
Pig	1–30 days	16–32	n.d.	164
	2 mths	19–25	22	163
	2–12 mths	14–37	n.d.	163
	1–2 yrs	18–35	n.d.	163
	2–6 yrs	22–36	n.d.	163
Rabbit (Chinchilla)	n.d.	1–7	5	304
Rabbit (New Zealand white)	n.d.	2–10	4	172
Rat	10 days	5–6	n.d.	227
	1 yr	15–18	n.d.	227
Sheep	6–8 mths	24–40	n.d.	164
	adult	32–35	n.d.	164

domestic animals [164]. The magnitude of development of PP appears to correlate in part with the degree of antigenic stimulation of intestinal mucosa [165,166]. Before puberty patch size varies in humans from a few millimeters to about 10 cm in length, and in the adult they can be as long as 28 cm. One PP can contain 5–250 lymphoid follicles, and as many as 980 have been identified.

Reynolds and coworkers [167] found evidence for the existence of two distinct types of PP in sheep. The ileocecal PP are dominated by lymphoid follicles that are tightly packed together, while the follicles in the PP in the jejunum are more pear-shaped and, in general, smaller than those in the ileocecal PP. The rabbit also has two different types of gut-associated lymphoid tissues. The rabbit appendix is similar to the ileocecal PP in sheep, while the PP in the rabbit intestine resemble the jejunal PP in sheep. Studies by Hein et al. [168] also supported the idea of different types of PP in lamb. Their results support the proposition that jejunal PP in lamb

are very similar to that found in other species, whereas the ileal PP are different and most likely have a specialized role in B-cell production similar to the bursa of Fabricius in birds. Peyer's patches are not present in all animals. There are no reports of PP in some primates such as *Mandrillus leucophaeeus* and *Papio anubis* [169]; PP have not been observed in cynomolgus monkey (unpublished observation). However, PP were located in Rhesus monkeys (*Macaca mulatta*) by means of a dissecting microscope [170].

In New Zealand white rabbits, the mean size of PP is about 0.7 cm^2 [171,172]. In these rabbits there are usually 40–50 nodules in a patch. Light microscopic studies have demonstrated that the nodules are separated from each other by intervening villi and interfollicular spaces. Each nodule is divided into three approximately equal zones (Fig. 13). According to Faulk et al. [172] the lower third of PP, toward the serosal surface, consists of large lymphocytes; the middle third consists of small lymphocytes; and the upper third, toward the epithelium, consists of a mixed cell population (Fig. 14). A study revealed the presence of collagenase in PP but not in adjacent ileum [172].

Peyer's patches host a wide spectrum of lymphocytes including B, T and dendritic cells. Lymphoid cells represent approximately 30% of the total cell population in the highest region of the dome FAE [173]. Follicles with germinal centers host mostly B lymphocytes, which are actively dividing, and distinct from non-germinal center B cells by their ability to bind high levels of peanut agglutinin [174]. T lymphocytes predominate in the parafollicular area and are also present in the dome region overlying PP. The origin of the T cells determines their phenotype. T cells derived from PP show a striking predominance of T8 ($CD8^+$) cells (cytotoxic/suppressor phenotype), and some cells located around postcapillary venules (PCV) express interleukin 2 (IL-2) receptors [175,176]. In adult mice 11–40% of PP lymphocytes are T cells, whereas 40–70% are B cells [177–179]. Similar numbers were found in New Zealand white rabbits and in children [180,181]. Biopsy specimens revealed that the mean cell yield was $1.7 \cdot 10^8$ lymphocytes/g tissue, and 26–48% were B cells, whereas T cells accounted for about 50%.

B cells from PP, when compared to B cells from peripheral lymph nodes, generate a greater proportion of B cell clones that produce only IgA [182,183]. Regulatory T cells from PP appear to have a selective role in switching from IgM to IgA commitment and have an amplifying role in the development of mature antigen-specific IgA B cells [184,185].

All mature, non-activated lymphocytes, independent of tissue source or subclass, can recirculate and reach almost all locations in the body [186]. However, distribution is not random. B cells preferentially migrate to mucosa-associated lymphoid tissue. This is clearly not true for effector cells nor presumably for antigen-specific memory cells. Once activated at specific sites of the body, the population of the immunoblasts and effector cells exhibit remarkable selective migratory behavior, moving mainly to tissues originally involved in antigen exposure and activation [187,188]. For example, B lymphocytes that exit PP appear to migrate to the mesenteric lymph nodes, the superior mesenteric duct, and the thoracic duct lymph before entering the circulation and returning to the lamina propria and intraepi-

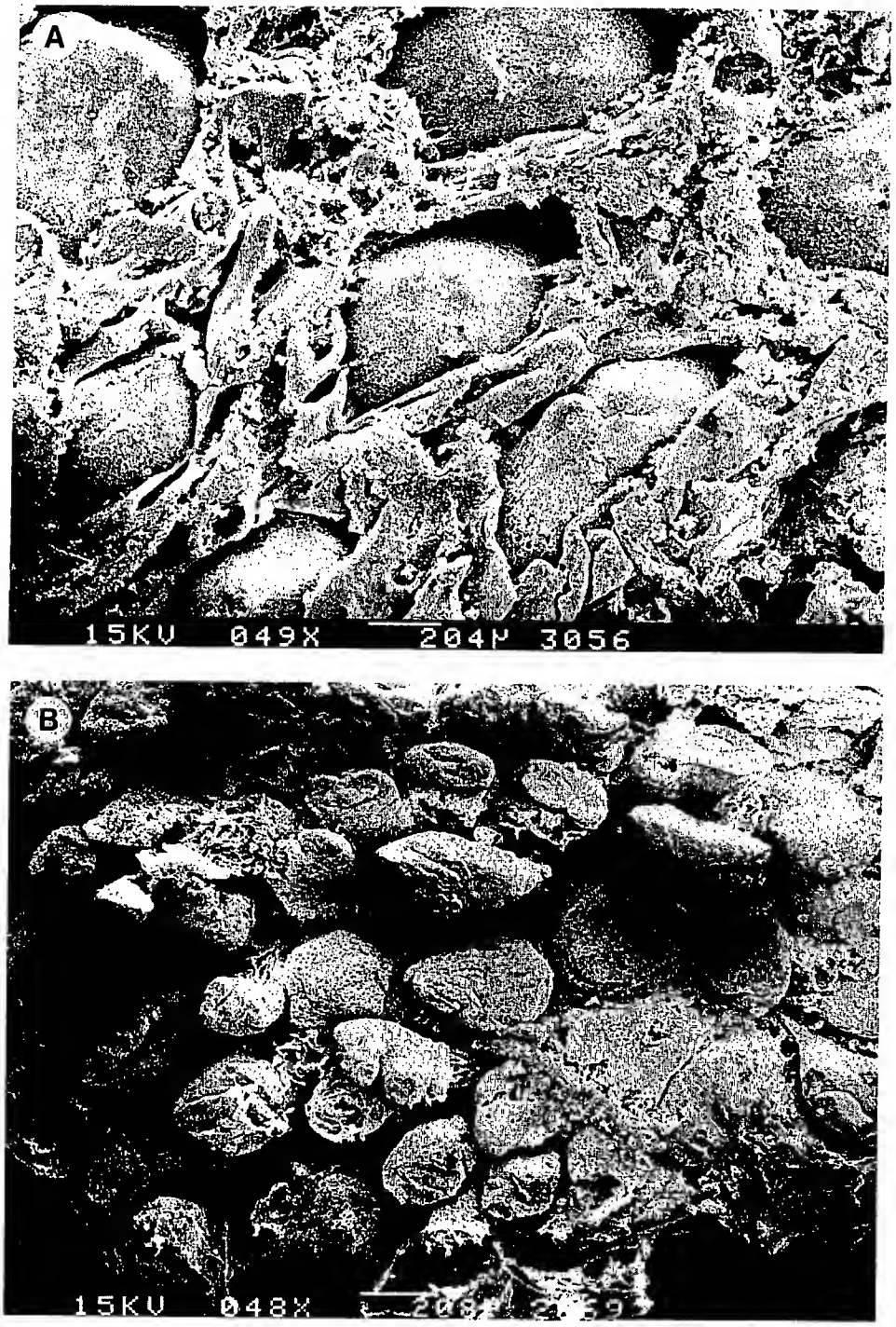

Fig. 13. Scanning electron microscopy of one (A) rabbit and (B) dog Peyer's patch.

Fig. 14. Cellular zones of dog Peyer's patches. LL, large lymphocyte zone; SL, small lymphocyte zone; MC, mixed cell zone.

thelial region of the PP via postcapillary vascular sites called HEV (Fig. 15) [177–193].

All secondary lymphoid tissues except spleen are permanently equipped with these specialized HEV. High-endothelial cells are, in contrast to other vascular endothelial cells, morphologically cubiodal, plump cells. Endothelial height seems to be related to the intensity of continuous passage of lymphocytes. HEVs are per-

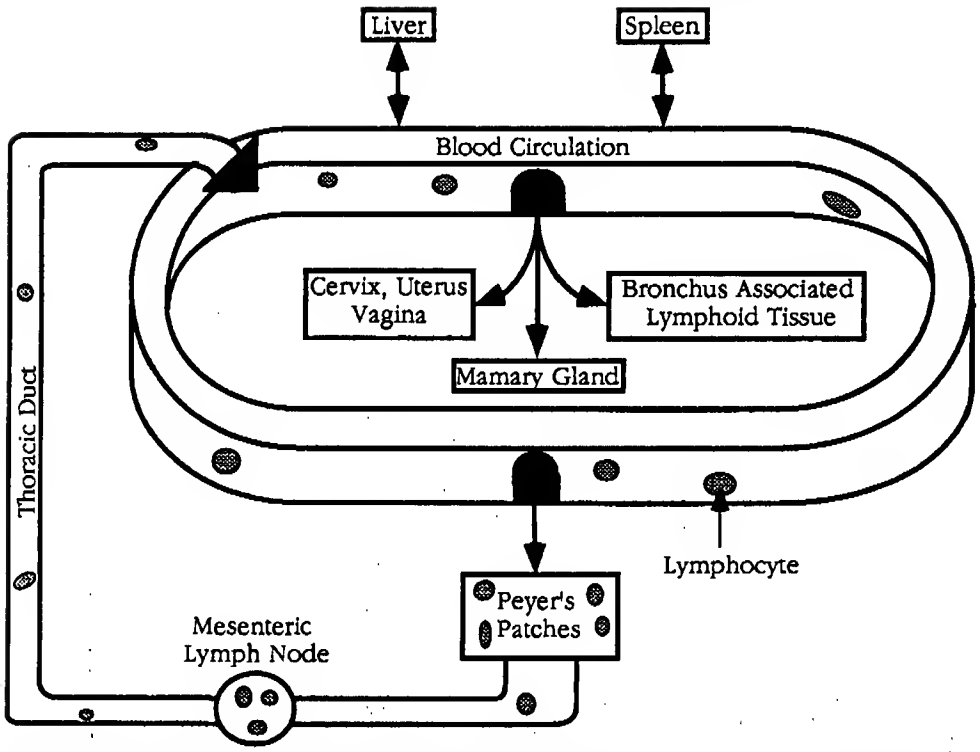

Fig. 15. Migration pathway of Peyer' patch cell. Lymphocytes from Peyer's patches enter the mesenteric lymph node and thoracic duct lymph before entering the circulation. These cells then disseminate to the lamina propria of the intestine and bronchus-associated lymphoid tissue. Cells from the mesenteric lymph nodes can disseminate also to extraintestinal sites such as the mammary gland, female genital tract, or bronchus-associated lymphoid tissue (from Refs. 193, 187).

manent in lymphoid tissues; however, their number and length seem variable and controlled by local immune activity. Antigenic stimulation results in a rapid increase in high endothelial vasculature [187].

High-endothelial venules host tissue-specific endothelial adhesion molecules (vascular addressins). The molecule has been identified as a 58–66 kDa protein and it is possible that it represents the ligand for lymphocyte homing receptors [187]. A calcium-dependent phosphomannosyl binding site has been linked to the recognition of peripheral lymph nodes in man, rat and mouse [194].

Lymphocytes have surface structures (homing receptors) which mediate interaction with the vascular addressins. For example, T lymphoblasts have two categories of homing receptors, those influenced by antigen stimulation and those determined by tissue differentiation [195]. In addition to homing receptors, which direct tissue-specific interaction of lymphocytes with HEV, a lymphocyte function-associated antigen (LFA–1), which does not discriminate between tissues, is involved in lymphocyte-endothelial interaction. LFA-1 is an accessory adhesion molecule in lymphocyte binding to HEVs. Most likely, primary recognition of HEVs by homing

receptors is followed by a cooperative action of homing receptors and LFA-1 in establishing stable cell contacts [187].

T lymphoblasts in PP also have a migration pattern similar or identical to that described for B cells [196]. Organ specificity is largely determined by selective interaction of circulating lymphocytes with endothelial cells of postcapillary venules [197]. Support for this theory came from Pabst and Reynolds [198] who examined the migration behavior of lymphocytes from PP. Their study in sheep demonstrated that lymphocytes emigrate from the gut and mesenteric lymph nodes via lymphatics, and do not enter the blood directly in large numbers as occurs in pigs [199]. The total number of FITC-labeled lymphocytes found throughout the sheep after 1 day was about 60 times greater after ileal PP perfusion with FITC than after jejunal PP perfusion, $100 \cdot 10^9$ and $1.6 \cdot 10^9$, respectively, although the mass of labeled PP was only 25 times greater after ileal perfusion than after jejunal PP perfusion, 107 g and 4.2 g, respectively. Thus, it was apparent that the number of FITC-labeled cells remote from the perfused site was not directly related to the amount of PP perfused. About 4 times more lymphocytes emigrated per g of perfused jejunal PP when compared to ileal patches, $72 \cdot 10^6$ and $17 \cdot 10^6$, respectively. Moreover, lymphocyte transport from the intestinal mucosa seems to be dependent on the absorption of nutrients. Miura et al. [200] showed that in situ lymphocyte transport from the intestinal mucosa was stimulated to more than a factor 10 by olive oil administration, suggesting that fat absorption could be an important factor influencing the lymphocyte transport in the lymphatic system in the intestine.

Mahida et al. [201] investigated the mononuclear phagocyte system within human PP. Macrophages in the germinal center were large, while macrophages in the dome region were heterogeneous in their size distribution. Staining for acid phosphatase was observed in both areas. In the parafollicular areas there were cells with a dendritic morphology, which did not stain for acid phosphatase. Some macrophages and lymphocytes in the germinal centers and dome regions expressed interleukin 2 (IL–2) and transferrin receptors.

Macrophages extending pseudopods into or entering the follicle epithelium supplement lymphocytes which take up macromolecules transported from the lumen by M cells and take up microorganisms, such as *Giardia muris* or *Mycobacterium paratuberculosis*, which are too big to pass through the M cell tubulovesicular system. Phagocytosis of microorganisms, in PP lymphoid follicles, provides a route for communication of antigenic information leading to the defense against the effects of further uncontrolled entrance of antigenic particles into PP lymphoid follicles [202,203].

In an attempt to describe the phagocytosis behavior of FAE, PP cells were incubated in vitro with FITC-polystyrene microspheres (0.75 μm) at a ratio of 100 particles/cell, which were then immunologically stained. Phagocytotic cells comprised 51–55% of the PP population, but only a subpopulation (15–25%) both phagocytosed FITC-microspheres and expressed the antigens required for recognition by the antibodies [204].

Hayakawa and Lee [205] investigated the enzymatic barrier of PP in the albino rabbit. The results indicate equal aminopeptidase activity in jejunal as in ileal PP, but

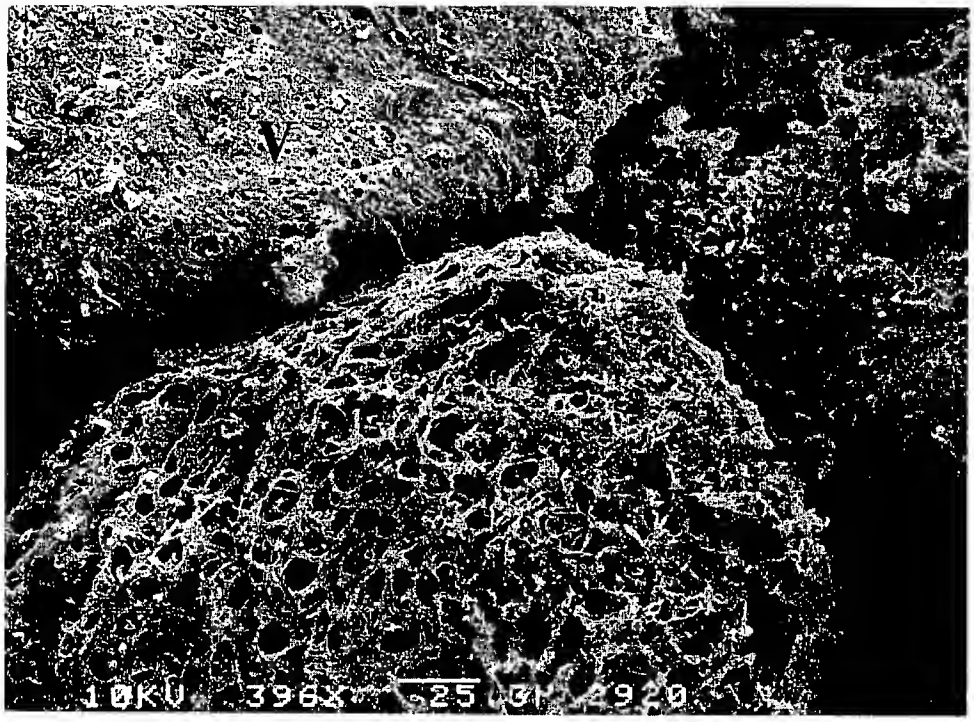

Fig. 16. Dog ileal Peyer's patch. Epithelial removal reveals the basement membrane. The pores in the basement membrane overlying the lymph node (LN) are greater than that of the villus (V).

activity was about one third when compared to neighboring epithelial columnar cells. Inhibition studies with several compounds such as amastatin, bestatin, *p*-chloromercuribenzoate, puromycin, sodium deoxycholate, sodium glycocholate and polyoxyethylene-9-lauryl ether in patch and non-patch tissue suggested that the aminopeptidases present in PP might be different in both type and activity than those in non-patch tissues.

Several authors [206–208] investigated the porosity of the epithelial basement membrane overlying lymphoid follicles within PP of rats and owl monkeys by scanning electron microscopy. Basement membranes overlying lymphoid follicles are markedly porous, and pore size increases centrifugally from the cap to the periphery of the follicle. There was no remarkable difference in porosity of the basement membrane within the terminal ileal segments of all animals. Additionally, the porosity of the basement membrane overlying the follicles is greater than that of the villus cores. Results from our investigations in beagle dogs confirm their findings (Fig. 16). One might speculate that such porosity could facilitate transcytosis of luminal macromolecules into the underlying follicles.

Microscopic examinations of the cells lying over PP follicles (dome region) have revealed that the relatively sparse number of goblet cells creates a breach in the mucus blanket allowing particles to reach the lympho-epithelium, consisting of a

single layer of specialized cells (M cells). Epithelial staining with ruthenium red confirmed that M cells have a less elaborate glycocalyx than absorptive cells [209]. These cells transport antigenic material from the lumen into the space between cells where it can be taken up by lymphocytes [210–213]. In this respect, PP can be viewed as the initiating site of mucosal immunity. However, the M cell, because of its lack of major histocompatibility complex (MHC) class II antigens can not function directly as an antigen-presenting cell [214]. It has been postulated that M cells in concert with absorptive enterocytes are present in the lamina epithelialis in every region where it overlies an accumulation of lymphoid tissue such as PP and/or solitary lymphoid follicles. These are located throughout the intestinal tract, particularly in the colon and rectum, with an average of 3–4 follicles per cm^2 in the large intestine [154,215,216]. These M cells were also identified in human and rabbit appendices and tonsils, the cecal PP of mice and rats [212–220], pigs [163], cows [221,222], dogs, hamsters [223], monkeys [224] and chickens [225].

The density of M cells is about 10–15% of the total cell population in the highest region of the dome FAE [220,226]; however, this statistic demonstrates a high inter-species variability. For example, in human PP the proportion is 1 M cell per 36 enterocytes [227], while in Wistar rats the ratio is 1 M cell per 12 enterocytes [213]. It has been estimated that in rabbits the M cells constitute about 30–50% of the total membrane area taken as a planar surface [228]. In mice, M cells are found to be distributed evenly throughout the higher regions of the FAE. The frequency decreases towards the lower regions and no M cells are found in the follicle-associated crypts. Antigen exposure stimulates the magnitude of PP development as well as the M cell number [229]. The transfer of specific pathogen-free (SPF) mice into a normal animal house environment increased the M cell area threefold within 7 days after the transfer. It was suggested from these results that M cell production can be selectively increased within the FAE through the presence of foreign antigens. The localization of antigens was investigated in duodenal, jejunal, and ileal PP, cecal patch, sacculus rotundus (posterior end of the rabbit ileum), and appendix. The antibodies labeled a subpopulation of FAE cells which comprise half of the epithelial cells. The immunostaining was mainly observed on FAE cell basolateral surfaces corresponding to the central hollow or pocket containing clusters of mononuclear leukocytes, which is characteristic of M cells. In contrast, the stem cell and proliferative regions facing the lamina propria were devoid of immunologically reactive sites. Under scanning electron microscope M cells in humans are characterized by luminal microfolds rather than microvilli (Fig. 17). M cells share tight junctions (TJ) and desmosomes with adjacent epithelial cells (absorptive enterocytes). Mean TJ strand counts and depths among adjacent absorptive cell pairs in guinea pigs ranged from five to seven strands and from 275 to 350 nm, respectively, while some TJ in follicular dome epithelium showed strand counts up to 16 and depths up to 600 nm [230]. The epithelium appears to consist of a reticulum formed by the attenuated cell processes of M cells, allowing lymphoid cells, such as lymphocytes, lymphoblasts, or macrophages, to approach to within 300 nm of the intestinal lumen, while maintaining the integrity of the intestinal epithelium [211,223]. Studies were conducted to describe and compare the morphology of M

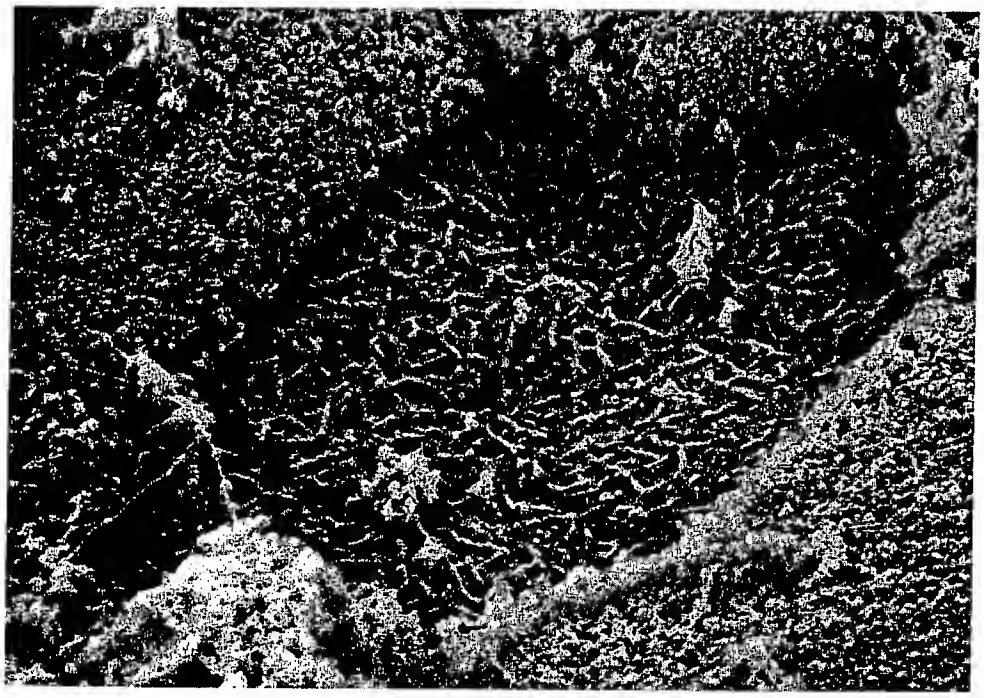

Fig. 17. Scanning electron micrograph of the surface of one M cell (from Ref. 211).

cells in both human and rat PP [227,231]. The basic structure of M cells in both humans and rats appears similar within the limits of transmission electron microscopy. Sicinski's results suggest the occurrence of two subtypes of M cells. However, in adult rats there were four types of surface structures. The difference was mainly due to the shape and number of microvilli sitting on the microfolds, with Type I having the most and Type IV no microvilli. An age difference was found in the ratio of the four cell types, with 10–20-day-old rats having 84% of Type I or II and only 5% of Type IV. After one year 35% of the M cells were of Type IV, and cells of Type I and II tended to be fewer in number. M cell renewal occurs by proliferation in multiple adjacent crypts with cell migration onto follicle surfaces [131, 232,233]. M cells have been shown to express Ia antigen and to synthesize interleukin-1 (IL-1) [234–236].

Several authors have found that PP are deficient in lysosomes [237–240]. Enzymatic histochemistry in the dome region (M cell) of different species revealed a lack of, or only minor activity of alkaline phosphatase, and little activity of acid phosphatase, succinate dehydrogenase, leucin-aminopeptidase, B-galactosidase [241]. Acid phosphatase was found in dense bodies in enterocytes but not in M cells. Comparison between enterocytes and M cells has shown that the volume fraction occupied by dense bodies in M cells is 16 times less than in enterocytes. Both the absence of acid phosphatase activity and the small number of dense bodies in M cells correlates with the absence of lysosomal degradation of luminal microor-

ganisms during transport into lymphoid follicles by M cells. The lack of alkaline phosphatase activity has been explained as a special characteristic of antigen-sampling epithelial cells. Furthermore, in contrast to absorptive cells, M cells lack any evidence for lipid absorption [131]. Observations from Madara et al. [230] revealed that M cell apical plasma membranes have a low protein-to-lipid ratio and an abundance of morphologically detectable cholesterol, except in domains involved in endocytosis.

Identical binding for concanavalin A, ricinus communis agglutinin, wheat germ agglutinin and peanut agglutinin on M cells and enterocytes indicates that the two cell types share the same common glycoproteins and glycolipids [219]. However, Neutra and coworkers [242] found that although ferritin-conjugated ricin binds in vitro at 4°C almost equally to both enterocyte and M cell surfaces, only M cells took up ricin and transported it to their basolateral membranes. Additionally, it was found that M cells transport cationized ferritin with an external diameter of 12–13 nm within vesicles, to the intercellular spaces [131–133]. The molecular weight of ferritin is in between that of apoferritin (450 kDa) and holoferritin (900 kDa) depending on the iron content. Simultaneous application of an adherent probe such as wheat germ agglutinin-ferritin (WGA-ferritin) and a non-adherent one such as BSA-colloidal gold revealed an average ratio of 100 WGA-ferritin to 1 BSA-gold in intracellular vesicles. Counts of both tracers in the abluminal M cell compartment demonstrated that WGA-ferritin and BSA-gold had been transcytosed in a ratio of about 195 to 1. The dome epithelium, composed of absorptive epithelial cells, M cells and lymphocytes, which covers GALT provides both a protective barrier over lymphoid follicles and a route for antigen entry into the host. IgA was noted to be the predominant immunoglobulin in external secretions, and to form a continuous coating over M cells, thereby protecting the host [243]. IgA secretion can be provoked by antigen challenge and exposure to (e.g., bacterial lipopolysaccharides), which will induce B cells in the PP to eventually develop into IgA plasma cells [244]. Using light microscopic autoradiography and electronmicroscopy, Weltzin and coworkers [245,246] found that all gold-labeled or radiolabeled mouse IgA was bound to M cells upon injection into mouse, rat, and rabbit intestinal loops containing PP. Competitive experiments with unlabeled IgA demonstrated inhibition; thus, binding was specific. The authors suggested that luminal antigens and microorganisms, complexed with secretory IgA (sIgA), could be targeted to M cell surfaces, and selectively transported to underlying macrophages and lymphoid cells. In vivo, the source of sIgA is intestinal secretion as well as bile [247]. The authors consider the immunologic functions of sIgA as threefold: (a) antigen exclusion; (b) antimicrobial functions; and (c) participation in cell-mediated immunity. Antigen exclusion could hamper oral vaccination; however, it is possible to prevent the formation of an sIgA-peptide/protein-drug complex by combining the drug with inert soluble polymers, which may preserve the therapeutic action of the active compound [248]. Pappo and Owen [249] examined the expression of the secretory component (SC), a transmembrane glycoprotein by GALT epithelial cells. SC has been shown to function as the specific binding receptor for dimeric IgA. They found that SC expression is absent on M cells and all other follicle epithelial cells. This result

seems to be in contrast to findings from Weltzin et al. [246], who found selective binding of IgA to M cells. However, they confirmed that M cells do not express the receptor for dimeric IgA [250,251]. At this time, it appears that the nature of the M cell membrane component(s) responsible for IgA adherence is still unknown.

III.2. Uptake into Peyer's patches

Transport across the epithelium in PP can be achieved by three distinct pathways: (i) endocytosis; (ii) via the paracellular shunt; and (iii) by passive diffusion across cells in PP, the latter being not of great importance due to the characteristic transport properties of this particular tissue. Endocytotic activity seems to depend on age and health state of the subject [252]. Several microorganisms can gain access to the circulation via receptor-mediated endocytosis in PP [253–255]. In vitro permeability measurements have been used to determine the comparative paracellular restriction in PP and non-patch tissues [171].

III.2(a) Adsorptive endocytosis. Non-receptor specific binding can result in internalization of solutes with subsequent transport through the cytoplasm. There are several substrates that recognize and bind to terminal sugars of glycolipids and glycoproteins. Among these are lectins and immunoglobulins. M cells and enterocytes, however, share the same common glycoproteins and glycolipids, thus a selective targeting of M cells is as yet not possible with lectins [219], and to date there appear to be no reports of successful targeting to M cells by lectins.

III.2(b) Fluid-phase endocytosis. Fluid phase endocytosis involves a non-selective uptake of solute molecules and has been demonstrated in rat PP [210]. Endocytosis can be investigated in vitro by permeability studies using a side-by-side diffusion chamber [256,257]. Several authors [239,258] have conducted in vitro transport studies, in Ussing chambers, using patch and non-patch jejunal tissues from 20-day-old piglets with and without PP, and HRP as the substrate. HRP is a 40 kDa protein with a molecular diameter of about 5 nm. By measuring the peroxidase activity, the mean HRP transport rate across intestinal segments with patch tissue was found to be increased threefold over non-patch tissue. However, Ducroc and coworkers [259] using the same substrate could not confirm these findings in 2–3 kg rabbits. No significant difference was found between the HRP fluxes through patch tissue and non-patch jejunal tissue. The authors assumed that there was a reduced degradation in PP when compared to non-patch tissue; however, this was not actually demonstrated in their study. The transport mechanism in both tissues was sensitive to metabolic inhibitors, thus, transcytosis is likely due to endocytotic processes.

The effect of oral immunization on the uptake of HRP by adult rat intestinal columnar epithelium and PP tissue in vitro has been investigated by Beahon and Woodley, by feeding rats daily with HRP for 14 days [260]. No significant difference between control and immunized animals for uptake rates into columnar epithelium was established. However, immunization decreased the uptake of intact HRP into PP by 60%, and transfer through patches into serosal fluid was 75% of

that for non-immunized animals. The same authors [261] failed to find a correlation between molecular weight and uptake rates into columnar epithelium and PP tissue in an everted gut-sac system, using HRP (M_r = 40000), bovine serum albumin (M_r = 68000), and IgG (M_r = 146000–165000). A comparison between precipitated protein and total radioactivity found revealed that degradation in PP was less than in non-patch tissue, confirming that there is less lysosome activity.

Heyman and coworkers [262] studied the intestinal absorption of an immunomodulating compound, which induces IL-1 secretion and activates other macrophage functions, in Ussing chambers using rabbit tissue. The compound is composed of two subunits of 350 and 95 kDa. Total transepithelial flux of tritium labeled compound (including degraded and non-degraded material) was 4.6-fold greater across the duodenum when compared to PP. Examination of the transported compounds revealed a higher degradation in non-patch tissues. Approximately 4% remained as a high molecular weight fraction after transport across the duodenum while about 17% was found to be the high molecular weight fraction after transport through PP.

III.2(c) Phagocytosis. Phagocytosis is the intake of large particles, e.g. beads up to several micrometers in diameter [126]. Particles measuring up to 35 μm and consisting of different materials such as carbon, latex, lycopodium spores, urtica pollens, and poly(DL-lactide-co-glycolide), have been shown to accumulate in PP of different species [188,213,263–272]. At no time were microspheres observed to penetrate at sites other than PP.

Size of the microparticles determines their fate in lymphoid tissue. Several authors have demonstrated the transport of microspheres of less than 5 μm in diameter through PP into macrophages within mesenteric lymph nodes [264,268,272]. This result is in agreement with the results of LeFevre and Joel [273] who indicated that the majority of particles which traverse PP are then sequestered into macrophages. In the case of antigen-loaded microspheres it is believed that particles up to 5 μm induce a predominantly circulating antibody response based on their propensity to migrate to systemic lymphoid tissues. On the other hand, microspheres of at least 5 μm are believed to trigger mainly a mucosal immune response because they remain in the IgA-inductive environment of the PP over the course of their antigen release.

It has been suggested that particle surface properties, as well as particle size, govern accumulation in PP, with penetration of the PP epithelium being more easily achieved by particles with hydrophobic surfaces than those with a hydrophilic character [272,274]. Similar results were obtained by Jani and coworkers [275] in rats. Uptake for non-ionic polystyrene microspheres appears higher than for a negatively charged type. Additionally, they also observed a relationship between size and uptake, with particles smaller than 1 μm (100 nm or 500 nm) being taken up at higher rates than particles with a diameter of 1 μm. In a follow-up study the same authors quantitated the extent of microsphere uptake [276]. Measurements of the polystyrene content yielded 35% uptake for particles of 50 nm and 25% for particles with 100 nm diameter. These results were challenged by De Keyser and co-

workers [277] who found that nanoparticles made from diethylmethylidenmalonate (DEMM) having a diameter between 140 and 250 nm were not absorbed in the gut. Although hydrophobic character was not assessed, one can assume that the surface is rather hydrophobic, due to their rapid blood clearance and fast onset of liver uptake after i.v. administration. Hydrophobicity is the major parameter for RES uptake in the liver and spleen [278].

Factors other than size and surface properties also seem to be of importance. Ebel found a relationship between the fed state of the subject and the maximum number of particles in PP and spleen [269]. Peyer's patches in fasted mice took up 30% less compared with fed mice. The mechanism for this observation is as yet unknown. It may be that the increased transit time in the gut or the presence of food increases the M cell uptake.

Additionally, the vehicle in which latex particles are administered alters their absorption behavior [279]. Following administration of latex particles in water to rats, the uptake during the first two hours was 2–6 times greater when compared to an emulsion consisting of arachis oil, water, Span 83 and Tween 80 (8:8:1:1). At 3 h no difference was observed. The supplementation of the emulsion with poloxamer 908 did not show any improvement compared to the emulsion alone.

The amount of particles administered has been related to the number of latex particles found in PP and in lymph-node tissue [280]. Particles remained in mice PP for 74 days after the cessation of feeding, with the number declining to about 10% of the original number observed. The total number of latex containing villi, surrounding a follicle, was small, about 3 or 4 out of an available 10–20 villi. In contrast to PP where latex formed aggregates, latex was usually isolated in villi. Latex was never observed in villi distant from PP [269,275]. The number of particles detected in PP apparently increases with increasing dose; however, there was no linear relationship to the dose administered.

Exposure of the subject to microorganisms may not be of mechanistic importance for PP uptake. No difference in transport pathway was determined, when absorption experiments were performed with latex particles (2 μm) in germ free mice, although non-germ free mice had significantly larger follicles, and therefore greater accumulation [281].

Not surprisingly, age seems to play a role in PP uptake. By counting carboxylated rhodamine B-conjugated polystyrene latex particles (negatively charged), LeFevre [266] demonstrated that the PP of aged mice take up about five times more latex (1.8 μm in diameter) than those of young mice. The authors suggest that this result is due to the slower transit of digested material through the small intestine of aged mice, thus, longer exposure leading to greater uptake. Alternatively, if one could speculate that the epithelial surface charge may change with age then this could affect the interaction with latex.

Since phagocytosis of particulate macromolecules has been shown to be greater than pinocytosis, several investigators have encapsulated antigens into unilamellar dipalmitoyl PC liposomes with a negative surface charge (for a review see Ref. 282) Michalek et al. [283] demonstrated M cell uptake and a successful immune response in rats from a liposomal antigen preparation. Recently Childers and cowork-

ers [284] injected gold-labeled solid core liposomes (AuSCL), made from dioleoyl PC/cholesterol/dioleoylphosphatidylglycerol/triolein (4.5:4.5:1:1) into isolated small intestinal sections of rats containing PP. After 2 h of incubation, sections were assayed for liposome uptake using thin section electron microscopy. M cells from the dome region of PP were found to have pinocytotic vesicles containing AuSCLs, supporting the assumption that liposomes can be taken up by M cells for induction of mucosal immune response.

Michalek et al. [285] also investigated an oral vaccine consisting of liposomes and antigen (*S. mutans*) in germfree Fischer rats. *S. mutans* whole cell antigen, when given orally, was effective in inducing a salivary IgA response. On the other hand, an oral vaccine consisting of purified *S. mutans* serotype CHO induced no immunological response. When the CHO was incorporated in liposomes, however, a good salivary IgA response was observed. Incorporation of muramyl dipeptide (MDP) also augmented the salivary response to CHO. The authors are currently studying the induction of mucosal responses in humans given an oral vaccine consisting of CHO in liposomes.

Most recently Rhalem et al. [286] incorporated the surface antigen of a sporozoite of *Eimeria falciformis* into liposomes. After intragastric administration, mice were then challenged with 400 oocysts of *E. falciformis* and the oocyst discharge was followed. The oocyst discharge for the treated mice was 36000/g on day 7, while in control mice the discharge on day 7 was 2100/g, suggesting that liposomal incorporation increased efficacy 10–15-fold.

III.2(d) Receptor mediated endocytosis. The epithelium of the gut expresses a variety of receptors for different ligands. Several microorganisms such as reovirus and poliovirus are known to selectively adhere and penetrate PP [287]. On the other hand liposomes are not transported intact across the intestinal epithelium [288]. Directing liposomes to the transcytosis pathway might be a promising approach to overcome this restriction, e.g., by linking the liposomes to a ligand that has a receptor in the gut. One approach, e.g., is the utilization of the M cell attachment protein from reovirus. Reovirus uptake occurs upon binding of the cell attachment protein (σ1), via its carboxylic residue, to the receptor on M cells [289–294]. The amino-terminal portion of σ1 protein reveals a hydrophobic region [295]. Therefore, Rubas et al. [296] incorporated the reovirus M cell attachment protein into the bilayer of soyphosphatidylcholine liposomes by the detergent dialysis method. Competition studies with reovirus on mouse fibroblasts (L929 cells) grown as a monolayer revealed selective binding to the reovirus receptor. After in vitro incubation at 4°C for 1 h, approximately a 10-fold greater amount of coated liposomes was found associated with rat PP when compared to uncoated liposomes (Fig. 18). The in vivo success of such a delivery system may depend upon the immunological status of the subject. Seifert and Sass [188] immunized rabbits against human gammaglobulin and then attempted oral absorption of human gammaglobulin. Non-sensitized animals absorbed 90% of the dose, while the immunized group absorbed only 60%. However, from their work it is not evident whether or not the gammaglobulin was taken up primarily by the lymphoid tissue.

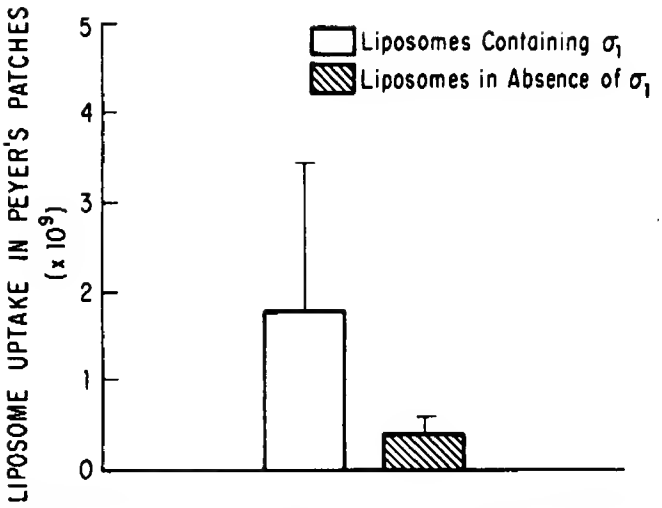

Fig. 18. Effect of in vitro incubation of rat Peyer's patches with either σ1 coated or uncoated liposomes on their uptake (from Ref. 296).

There are other microorganisms that could be potential sources for ligands to receptors on the M cell. Using electron microscopy, Sicinski and coworkers [253] demonstrated that after in vitro incubation of human PP with Sabin or Mahoney strain poliovirus (belonging to the same family as reovirus), virus particles adhere specifically to and are endocytosed by M cells. Poliovirus particles were found in coated pits, in coated vesicles, and in endosomes in the cytoplasm of M cells. The authors concluded that M cells are the site of poliovirus penetration of the intestinal epithelial barrier, from which the virus reaches the lymphatic tissue and systemic circulation.

In a similar experiment, ileal loops (8–10 cm) of non-immune rabbits were exposed to both virulent and avirulent shigellae. M cells contained approximately four times greater numbers ($P < 0.01$) of the pathogenic strain than the non-pathogenic strains. Heat-killed shigellae of the virulent strain were taken up by M cells to the same degree as the avirulent strains. However, the killed shigellae were unable to elicit a memory mucosal response, whereas non-pathogenic strains did, implying that there was heat destruction of epitopes necessary for stimulation [254]. These findings are, in part, different from the results of a study by Owen and coworkers [297]. For these researchers, inoculating a loop of non-immune rabbit with viable and killed *Vibrio cholerae* resulted in only the viable microorganisms being taken up by M cells. In an other study, Walker et al. [298] exposed small intestinal loops of rabbits to campylobacter. They found campylobacters in proximity to the mucosal surface of the ileum, however, when PP were examined, the bacteria were seen selectively associated with M cells. Bacteria were rapidly transported to lymphoid cells, presumably by macrophages. In this study, however, immunization was not investigated.

In addition to the above-mentioned microorganisms, various strains of Salmon-

ella were proven to colonize PP of mice [299]. Orally infected animals were assayed at various times after administration of bacteria for the number of microorganisms in PP. The bacterial counts rose to a peak about 6 days after infection and then gradually declined. The peak count was related to the amount of bacteria given. The invasion of PP induced resistance to oral challenge with virulent *S. typhimurium*. In order to study immune response in humans, Kantele et al. [255] exposed volunteers to an oral preparation of S. typhi strain Ty 21a and assayed blood, stool, and saliva for specific antibody-secreting cells (ASCs). ASCs were found in eight of ten subjects after the third postvaccination day, and the amounts peaked on day 7, but were undetectable on day 14. 62% of these ASCs produced IgA, 32% IgM, and 6% IgG, respectively. No immunoglobulins could be detected in serum, saliva or feces. The authors explain these findings as insufficient persistence of the antigen in the intestinal tract, which is needed to maintain local antibody production [300].

III.2(e) Paracellular restriction. The paracellular pathway is restricted by occluding junctions between adjacent cells and it is of interest to know if they behave similarly in patch and non-patch tissue. Permeability studies in PP from beagle dogs with poly(ethyleneglycol) (PEG) 900, PEG 4000, and mannitol suggest that there is no significant difference in paracellular restriction between PP and non-patch tissue [171]. Measured permeability declined similarly with increasing molecular weight in both patch and non-patch tissue (Table III). This is consistent with studies performed in 2-week-old piglets [258]. In contrast to piglets and beagle dogs, however, there was a difference observed between patch and non-patch permeability in rabbits using the same hydrophilic compound series (Table III). It was suggested that the difference is due to the difference in the number of intervening villi, which is much smaller for rabbits, thus, the total dome surface area and the M cell count in rabbit PP is much higher than in beagle dogs. The brush border at the apical pole of the enterocyte consists of 1500 to 3000 microvilli per cell, while M cells host microfolds rather than microvilli, thus, the mucosa surface is decreased by a factor of 15–25 [301]. Alternatively, such a difference may be due to the increased paracellular restriction through adjacent tight junctions with high strand counts and unusual depths [230].

TABLE III

PERMEABILITY FOR PASSIVELY TRANSPORTED MANNITOL, PEG 900, AND PEG 4000 IN BEAGLE DOG AND RABBIT PATCH AND NON-PATCH JEJUNAL SEGMENTS
Number in parentheses represents number of experiments

Compound	Permeability (10^{-6} cm/s)			
	beagle dog		rabbit	
	non-patch	patch	non-patch	patch
Mannitol	0.5500 ± 0.2578 (4)	0.4200 ± 0.2300 (8)	3.54 ± 0.86 (20)	2.52 ± 1.07 (14)
PEG 900	0.1600 ± 0.2209 (14)	0.1000 ± 0.0800 (7)	0.70 ± 0.03 (15)	0.19 ± 0.06 (9)
PEG 4000	0.0073 ± 0.0048 (3)	0.0044 ± 0.0045 (3)	0.24 ± 0.14 (15)	0.01 ± 0.01 (6)

Ho and coworkers [228] investigated the in situ structure absorption relationships of small compounds in rabbit patch and non-patch tissue. The authors observed an average permeability coefficient between $45 \cdot 10^{-5}$ and $50 \cdot 10^{-5}$ (cm/s) for the rapidly absorbed solutes *n*-octanoic, salicylic and taurocholic acids, and D-glucose. These in situ results demonstrated no difference between patch and non-patch tissue for the permeability of small molecular compounds.

III.2(f) Conclusion regarding absorption via lymphoid tissue. The metabolic capacity, in terms of peptide degradation, is lower in PP than non-patch tissue as determined by aminopeptidase activity and permeability studies. From the above discussion it is evident that the most relevant uptake mechanism for particles or microspheres in PP is phagocytosis. The extent of uptake depends on: (a) the surface properties, where a hydrophobic surface is favored; (b) the amount administered; and (c) the vehicle in which the particles are administered, with water being the best suspension vehicle. The fate after uptake in PP is determined by their size. Particles up to 5 μm exit the site of entry and migrate into lymphatic tissues such as lymph nodes, and spleen. Large particles of at least 5 μm remain in PP. All other endocytotic pathways are of minor importance, if present at all. Regarding the paracellular permeability of PP it seems that it is probably not different from non-patch tissue. Several microorganisms have a special M cell attachment protein that may be a tool for active targeting. Successful oral immunization is probably a result of the migration pattern of lymphocytes in PP.

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References

1 Roth, H.J., Dannhardt, G. and Lehmann, J. (1985) Seminar I: polymere Wirk- und Hilfsstoffe des modernen Arzneischatzes, Pharm. Z. 41, 2580–2582.
2 Parrot, D.M.V. (1976) The gut as a lymphoid organ, Clin. Gastroenterol. 5, 211–228.
3 Barrowman, J.A. (1978) Transport of absorbed lipids. In: J.A. Barrowman (Ed.), Physiology of the Gastrointestinal Lymphatic System, Cambridge University Press, Cambridge, pp. 165–213.
4 Granger, D.N. (1981) Intestinal microcirculation and transmucosal fluid transport, Am. J. Physiol. 240, G343–G349.
5 Granger, D.N. and Kvietys, P.R. (1984) Digestive system: small and large intestines. F. Lymphatic system. In: D.I. Abramson and P.B. Dobrin (Eds), Blood Vessels and Lymphatics in Organ Systems, Academic Press, Orlando, FL, pp. 450–455.
6 Kalima, T.V. (1981) The structure and function of intestinal lymphatics and the influence of impaired lymph flow on the ileum in rats, Scand. J. Gastroenterol. 6 (Suppl. 10), 9–87.
7 Kvietys, P.R., Wilborn, W.H. and Granger, D.N. (1981) Effects of net transmucosal volume flux on lymph flow in the canine colon, Gastroenterology 81, 1080–1090.
8 Granger, D.N. and Taylor, A.E. (1980) Permeability of intestinal capillaries to endogenous macromolecules, Am. J. Physiol. 238, H457–H464.

9 Clementi, F. and Palade, G.E. (1969) Intestinal capillaries. I. Permeability to peroxidase and ferritin, J. Cell. Biol. 41, 33–58.
10 Leak, L.V. (1976) The structure of lymphatic capillaries in lymph formation, Fed. Proc. 35, 1863–1871.
11 Granger, D.N., Miller, T., Allen, R., Parker, R.E., Parker, J.C. and Taylor, A.E. (1979) Permselectivity of the liver blood–lymph barrier to endogenous macromolecules, Gastroenterology 77, 103–109.
12 Comper, W.D. and Laurent, T.C. (1978) Physiologic function of connective tissue polysaccharides, Physiol. Rev. 58, 255–315.
13 Granger, D.N., Mortillaro, N.A., Kvietys, R., Rutili, G., Parker, J.C. and Taylor, A.E. (1980) Role of the interstitial matrix during intestinal volume absorption, Am. J. Physiol. 238, G183–G189.
14 Bierman, H.R., Byron, R.L., Kelly, K.H., Gilfillan, R.S., White, L.P., Freeman, N.E. and Petrakis, N.L. (1953) The characteristics of thoracic duct lymph in man, J. Clin. Invest. 32, 637–650.
15 Mortillaro, N.A. and Taylor, A.E. (1976) Interaction of capillary and tissue forces in the cat intestine, Circ. Res. 39, 348–358.
16 Lee, J.S. (1984) Lymphatic contractility. In: A.P. Shepherd and D.N. Granger (Eds.), Physiology of the intestinal circulation, Raven Press, New York, pp. 201–210.
17 Granger, H.J. (1981) Physicochemical properties of the extracellular matrix. In: A.R. Hargens (Ed.), Tissue Fluid Pressure and Composition, Williams and Willkins, Baltimore, pp. 51–61.
18 Granger, D.N. and Taylor, A.E. (1978) Effects of solute-coupled transport on lymph flow and oncotic pressures in cat ileum, Am. J. Physiol. 235, E429–E436.
19 Turner, S.G. and Barrowman, J.A. (1977) Intestinal lymph flow and lymphatic transport of protein during fat absorption, J. Exp. Physiol. 62, 175–180.
20 Granger, D.N., Perry, M.A., Kvietys, P.R. and Taylor A.E. (1982) Permeability of intestinal capillaries: effect of fat absorption and gastrointestinal hormones, Am. J. Physiol. 242, G194–G201.
21 Palade, G.E., Simionescu, M. and Simionescu, N. (1979) Structural aspects of the permeability of the microvascular endothelium, Acta Physiol. Scand. 463 (Suppl.), 11–32.
22 Kedem, O. and Katachalsky, A. (1958) Thermodynamic analysis of the permeability of biological membranes to non-electrolytes, Biochim. Biophys. Acta 27, 229–246.
23 Granger, D.N., Perry, M.A., Kvietys, P.R. and Taylor A.E. (1981) Interstitium-to-blood movement of macromolecules in the absorbing small intestine, Am. J. Physiol. 241, G31–G36.
24 Vaerman, J.-P. and Heremans, J.F. (1970) Origin and molecular size of immunoglobulin-A in the mesenteric lymph of the dog, Immunology 18, 26–38.
25 Vaerman, J.-P., André, C., Bazin, H. and Heremans, J.F. (1973) Mesenteric lymph as a major source of serum IgA in guinea pigs and rats, J. F. Eur. J. Immunol. 3, 580–584.
26 Casley-Smith, J.R. (1962) The identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals, J. Cell Biol. 15, 259–277.
27 Dobbins, W.O. and Rollins, E.L. (1970) Intestinal mucosal lymphatic permeability: an electron microscopic study of endothelial vesicles and cell junctions, J. Ultrastruct. Res. 33, 29–59.
28 Sabesin, S.M. and Frase, S. (1977) Electron microscopic studies of the assembly, intracellular transport, and secretion of chylomicrons by rat intestine, J. Lipid. Res. 18, 496–511.
29 Tso, P., Pitts, V. and Granger, D.N. (1985) Role of lymph flow in intestinal chylomicron transport, Am. J. Physiol. 249, G21–G28.
30 Jacobs, F.A. and Largis, E.E. (1969) Transport of amino acids via the mesenteric lymph duct in rats, Proc. Soc. Exp. Biol. Med. 130, 692–696.
31 Peters, T.J. and MacMahon (1970) Absorption of glycine and glycine oligopeptides by the rat, Clin. Sci. 39, 811–821.
32 Alexander, H.L., Shirley, K. and Allen, D. (1936) The route of ingested egg white to the systemic circulation, J. Clin. Invest. 15, 163–167.
33 May, A.J. and Whaler, B.C. (1958) The absorbtion of *Clostridium botulinum* type A toxin from the alimentary canal, Br. J. Exp. Pathol. 39, 307–316.
34 Warshaw, A.L. and Walker, W.A. (1974) Intestinal absorption of intact antigenic protein, Surgery 76, 495–499.
35 Katayama, K. and Fujita, T. (1972) Studies on biotransformation of elastase. II. Intestinal absorption of ^{131}I-labeled elastase in vivo, Biochim. Biophys. Acta 288, 181–190.

36 Katayama, K. and Fujita, T. (1972) Studies on biotransformation of elastase. I. Transport of ^{131}I-labeled elastase across rat intestine in vitro, Biochim. Biophys. Acta 288, 172–180.
37 Fortier, M., Bonfils, A., Zalisz, R., Heyman, M. and Smets, P. (1989) Pharmacokinetic profile of RU 41740, a bacterial immunomodulator, in mice, rats, and monkeys, Int. J. Pharm. 52, 27–36.
38 Yamashita, A., Ohtsuka, H. and Maeda, H. (1983) Intestinal absorption and urinary excretion of antitumor peptidomannan KS-2 after oral administration in rats, Immunopharmacology 5, 209–220.
39 Yuzuriha, T., Karayama, K. and Fujita, T. (1975) Studies on biotransformation of lysozyme. I. Preparation of labeled lysozyme and its intestinal absorption, Chem. Pharm. Bull. 23, 1309–1314.
40 Volkenheimer, G. and Schulz, F.H. (1968) The phenomenon of persorption, Digestion 1, 213–218.
41 Volkenheimer, G., Schulz, F.H., Hofmann, I., Pioeser, J., Rack, O., Reichelt, G., Rothenbaecher, W., Schmelich, G., Schurig, B., Teicher, G. and Weiss, B. (1968) The effect of drugs on the rate of persorption, Pharmacology 1, 8–14.
42 Volkenheimer, G. and Schulz, F.H. (1969) Effect of caffeine on the rate of persorption, Nutr. Dieta 11, 13–22.
43 Volkenheimer, G., Schulz, F.H., John, H., Meier Zu Eisen, J. and Niederkorn, K. (1969) Persorbed food particles in the blood of new-borns, Gynecologia 168, 86–92.
44 Volkenheimer, G. (1975) Hematogenous dissemination of ingested polyvinyl chloride particles, Ann. NY Acad. Sci. 246, 164–171.
45 Volkenheimer, G. (1977) Persorption of particles: physiology and pharmacology, Adv. Pharmacol. Chemother. 14, 163–187.
46 Volkenheimer, G. and Schulz, F.H. (1968) The effect of drugs on the rate of persorption, Pharmacology 1, 8–14.
47 Phillips, T.E., Phillips T.L. and Neutra M.R. (1987) Macromolecules can pass through occluding junctions of rat ileal epithelium during cholinergic stimulation, Cell Tissue Res. 247, 547–554.
48 Phillips, T.E., Phillips T.L. and Neutra M.R. (1984) Regulation of intestinal goblet cell secretion. IV. Electrical field stimulation in vitro. Am. J. Physiol. 247, G682–G687.
49 Krause, W., Mätheis, H. and Wulf, K. (1969) Fungaemie and funguria after oral administration of *Candida albicans*, Lancet 1, 598–599.
50 Stone, H.H., Kolb, L.D., Currie, C.A., Geheber, C.E. and Cuzzell, J.Z. (1974) Candida sepsis: pathogenesis and principles of treatment, Ann. Surg. 179, 697–711.
51 Aprahamian, M., Humbert, C., Balboni, G., Andrieu, V. and Devissaguet, J.P. (1986) Les vecteurs colloidaux polymériques (nanocapsules) franchissentils la barriére intestinal. In Proceedings of the International Conference on Pharmacology Technology, 4th edn., pp. 175–182.
52 Damgé, C., Aprahamian, M., Balboni, G., Hoetzel, A., Andrieu, V. and Devissaguet, J.P. (1987) Polyalkylcyanoacrylate nanocapsules increase the intestinal absorption of a lipophilic drug, Int. J. Pharm. 36, 121–125.
53 Damgé, C., Michel, C., Aprahamian, M. and Couvreur, P. (1986) Advantage of a new colloid drug delivery system in the insulin treatment of streptozotocin-induced diabetic rat, Diabetologia 29, 531 A.
54 Kreuter, J., Müller, U. and Munz, K. (1989) Quantitative and microautoradiographic study on mouse intestinal distribution of polycyanoacrylate nanoparticles, Int. J. Pharm. 55, 39–45.
55 Damgé, C., Michel, C., Aprahamian, M. and Couvreur, P. (1988) New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier, Diabetes 37, 246–251.
56 Eldem, T. and Speiser, P. (1989) Intestinal fat absorption and its related relevance in lipid drug delivery systems, Pharmazie 44, 444–447.
57 Muranishi, S. (1985) Modification of intestinal absorption of drugs by lipoidal adjuvants, Pharm. Res. 2, 108–118.
58 Palin, K.J. (1985) Lipids and oral drug delivery, Pharm. Int. 6, 272–275.
59 Cheema, M., Palin, K.J. and Davis, S.S. (1987) Lipid vehicles for intestinal lymphatic drug absorption, J. Pharm. Pharmacol. 39, 55–56.
60 Melone, J. and Mei, N. (1991) Intestinal effects of the products of lipid digestion on gastric electrical activity in the cat, Gastroenterology 100, 380–387.
61 Gowan, W.G. and Stavchansky, S. (1986) The effect of solvent composition upon the blood and lymph levels of phenytoin in rats after gastric administration, Int. J. Pharmaceutics 28, 193–199.
62 Hayashi, H., Fujimoto, K., Cardelli, J.A., Nutting, D.F., Bergstedt, S. and Tso, P. (1990) Fat feed-

ing increases size, but not number, of chylomicrons produced by small intestine, Gastrointest. Liver Physiol. 22, G709–G719.
63 Tso, P., Drake, D.S., Black, D.D. and Sabesin, S.M. (1984) Evidence for separate pathways of chylomicron very low density lipoprotein assembly and transport by rat small intestine, Am. J. Physiol. 247, G599–G610.
64 Ikeda, I., Imaizumi, K. and Sugano, M. (1987) Absorption and transport of base moieties of phosphatidylcholine and phosphatidylethanolamine in rats, Biochim. Biophys. Acta 921, 245–253.
65 Patton, J.S. and Carey, M.C. (1979) Watching fat digestion, Science 204, 145–148.
66 Patton, J.S. (1981) Gastrointestinal lipid digestion. In: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, New York, pp. 1123–1146.
67 Shiau, Y.-F. (1987) Lipid digestion and absorption. In: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, New York, pp. 1527–1556.
68 Rigler, M.W. and Paton, J.S. (1983) The production of liquid crystalline product phases by pancreatic lipase in the absence of bile salts. A freeze-fracture study, Biochim. Biophys. Acta 751, 444–454.
69 Larsson, K. (1972) On the structure of isotropic phases in lipid water systems, Chem. Phys. Lipids 9, 181–195.
70 Luzzati, V., Tardieu, A., Gulik-Krzywicki, T., Rivas, E. and Reiss-Husson, F. (1968) Structure of the cubic phases of lipid-water systems, Nature 220, 485–488.
71 Larsson, K., Fontell, K. and Krog, N. (1980) Structural relationship between lamellar, cubic and hexagonal phases in monoglyceride-water systems. Possibility of cubic structures in biological systems, Chem. Phys. Lipids 27, 321–328.
72 Tilcock, C.P. and Fisher, D. (1982) Interactions of glycerol monooleate and dimethylsulphoxide with phospholipids. A differential scanning calometry and ^{31}P-NMR study, Biochim. Biophys. Acta 685, 340–346.
73 Longley, W. and McIntosh, T. (1983) A bicontinuous tetrahedral structure in a liquid-crystalline structure, Nature 303, 612–614.
74 Arvidson, G., Brentel, I., Lindblom, G. and Fontell, K. (1985) Phase equilibria in four lysophosphatidylcholine/water systems. Exceptional behaviour of 1-palmitoyl-glycerophosphocholine, Eur. J. Biochem. 152, 753–759.
75 Brentel, I., Selstam, E. and Lindblom, G. (1985) Phase equilibria of mixtures of plant galactolipids. The formation of a bicontinuous phase, Biochim. Biophys. Acta 812, 816–826.
76 Rilfors, L., Eriksson, P.-O., Arvidson, G. and Lindblom, G. (1986) Relationship between three-dimensional arrays of 'lipidic particles' and bicontinuous cubic lipid phases, Biochemistry 25, 7702–7711.
77 Ericsson, P.-O., Lindblom, G. and Arvidson, G. (1987) NMR studies of micellar aggregates in 1-acyl-*sn*-glycerophosphocholine systems. The formation of a cubic liquid crystalline phase, J. Phys. Chem. 91, 846–853.
78 Sjölund, M., Rilfors, L. and Lindblom, G. (1989) Reversed hexagonal phase formation in lecithin-alkane-water systems with different acyl chain unsaturation and alkane length, Biochemistry 28, 1323–1329.
79 Gulik-Krzywicki, T., Aggerbeck, L.P. and Larsson, K. (1984) The use of freeze-etching electron microscopy for phase analysis and structure determination of lipid systems. In: K.L. Mittal and B. Lindman (Eds.), Surfactants in solution, Vol. 1, Plenum Press, New York, pp. 237–257.
80 Ericsson, B., Larsson, K. and Fontell, K. (1983) A cubic protein-monoolein-water phase, Biochim. Biophys. Acta 729, 23–27.
81 Engel, R.H., Riggi, S.J. and Fahrenbach, M.J. (1968) Insulin: intestinal absorption as water-in-oil-in-water emulsions, Nature 219, 856–857.
82 Shichiri, M., Shimizu, Y., Kawamori, R., Fukuchi, M., Shigeta, Y. and Abe, H. (1974) Eneteral absorption of water-in-oil-in-water insulin emulsions in rabbits, Diabetologica 10, 317–321.
83 Shichiri, M., Kawamori, R., Yshida, M., Etani, N., Hoshi, M., Izumi, K., Shigeta, Y. and Abe, H. (1975) Short-term treatment of alloxan-diabetic rats with intrajejunal administration of water-in-oil-in-water insulin emulsions, Diabetes 24, 971–976.
84 Hori, R., Okumura, K., Inui, K., Nakamura, N., Miyoshi, A. and Suyama, T. (1977) Pharmaceutical approach to the oral dosage form of macromolecules: effect of bile salts and oil-in-water emulsions on the intestinal absorption of urogastrone in the rat, Chem. Pharm. Bull. 25, 1974–1979.

85 Yanagawa, A., Iwayama, T., Saotome, T., Shoji, Y., Takano, K., Oka, H., Nakagawa, T. and Mizushima, Y. (1989) Selective transfer of cyclosporin to thoracic lymphatic systems by the application of lipid microspheres, J. Microencapsulation 6, 161–164.
86 Cho, Y.W. and Flynn, M. (1989) Oral delivery of insulin, Lancet 30, 1518–1519.
87 Cho, Y.W. and Flynn, M.J. (1990) Pharmaceutical Formulations, WO 90/03164.
88 Liedtke, R.K., Suwelack, K. and Karzel, K. (1990) Wirkung peroraler und transdermaler Insulin-Präparationen auf die Blutglukose-Konzentration bei Maüsen, Arzneim.-Forsch./Drug Res. 40, 880–883.
89 Clark, B. (1978) Chylomicron composition during duodenal triglyceride and lecithin infusion, Am. J. Physiol. 235, E183–E190.
90 Feldman, E.B., Russel, B.S., Chen, R., Johnson, J., Forte, T. and Clark, B.S. (1983) Dietary saturated fatty acid content affects lymph lipoproteins: studies in the rat, J. Lipid Res. 24, 967–976.
91 Fraser, R. (1970) Size and lipid composition of chilomicron of different Svedberg units of flotation, J. Lipid Res. 11, 60–63.
92 McDonald, G.B., Saunders, D.R., Weidman, M. and Fisher, L. (1980) Portal venous transport of long-chain fatty acids absorbed from rat intestine, Gastrointest. Liver Physiol. 2, G141–G150.
93 Tso, P., Lindström, M.B. and Borgström, B. (1987) Factors regulating the formation of chylomicrons and very-low-density lipoproteins by the rat small intestine, Biochim. Biophys. Acta 922, 304–313.
94 Bernard, A., Echinard, B. and Carlier, H. (1987) Differential intestinal absorption of two fatty acid isomers: elaidic and oleic acids, Am. J. Physiol. 253, G751–G759.
95 Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969) Very low density lipoproteins in intestinal lymph: role in triglyceride cholesterol transport during fat absorption, J. Clin. Invest. 48, 2367–2373.
96 Adjei, A.L., Cheskin, H.S., Vadnere, M.K. and Johnson, E. (1990) Pharmaceutical composition for oral administration, WO 90/08537.
97 Small, D.M. (1968) A classification of biologic lipids based upon their interaction in aqueous systems, J. Am. Oil Chem. Soc. 45, 108–119.
98 Ritschel, W.A., Ritschel, G.B., Sabouni, A. Wolochuk, D. and Schroeder, T. (1989) Study on the peroral absorption of the endekapeptide cyclosporine A, Methods Find. Exp. Clin. Pharmacol. 11, 281–287.
99 Ritschel, W.A., Adolph, S., Ritschel, G.B. and Schroeder, T. (1990) Improvement of peroral absorption of cyclosporine A by microemulsions, Methods Find. Exp. Clin. Pharmacol. 12, 127–134.
100 Ritschel, W.A. (1991) Microemulsions for improved peptide absorption from the gastrointestinal tract, Methods Find. Exp. Clin. Pharmacol., in press.
101 Rubin, C.E. (1966) Electron microscopic studies of triglyceride absorption in man, Gastroenterology 50, 65–77.
102 Porter, H.P., Saunders, D.R., Tytgat, G., Brunser, O. and Rubin, C.E. (1971) Fat absorption in bile fistula man. A morphological and biochemical study, Gastroenterology 60, 1008–1019.
103 Takada, K., Oh-hashi, M., Furuya, Y., Yoshikawa, H. and Muranishi, S. (1989) Enteric solid dispersion of cyclosporin A (CiA) having potential to deliver CiA into lymphatics, Chem. Pharm. Bull. 37, 471–474.
104 Yoshikawa, H., Takada, K. and Muranishi, S. (1984) Molecular weight dependence of permselectivity to rat small intestinal blood-lymph barrier for exogenous macromolecules absorbed from lumen, J. Pharm. Dyn. 7, 1–6.
105 Yoshikawa, H., Muranishi, S., Sugihara, N. and Sezaki, H. (1983) Mechanism of transfer of bleomycin into lymphatics by a bifunctional delivery system via lumen of small intestine, Chem. Pharm. Bull. 31, 1726–1732.
106 Pocock, D.M.E. (1974) DDT absorption and chylomicron transport in rat, Lipids 9, 374–381.
107 Muranishi, S., Takada, K., Yoshikawa, H. and Murakami, M. (1986) Enhanced absorption and lymphatic transport of macromolecules via the rectal route. In: S.S. Davis, L. Illum and E. Tomlinson (Eds.), Delivery Systems for Peptide Drugs, Plenum Press, New York, pp. 177–189.
108 Kaji, Y., Uekama, K., Yoshikawa, H., Takada, K. and Muranishi, S. (1985) Selective transfer of 1-hexylcarbamoyl-5-fluorouracil into lymphatics by combination of β-cyclodextrin polymer com-

plexation and absorption promoter in the rat, Int. J. Pharm. 24, 79–89.
109 Smith, R.N., Hansch, C. and Ames, M.M. (1975) Selection of reference partitioning system for drug design work, J. Pharm. Sci. 64, 599–606.
110 Austel, V. and Kutter, E. (1983) Absorption, distribution and metabolism of drugs. In: J.G. Topless (Ed.), Quantitative Structure-Activity Relationships of Drugs, Academic Press, New York, pp. 437–496.
111 Leo, A., Hansch, C. and Elkins, D. (1977) Partition coefficients and their uses, Chem. Rev. 71, 525–616.
112 Dainty, J. and House, C.R. (1966) Unstirred layers in frog skin, J. Physiol. 182, 66–78.
113 Ho, N.F.H., Park, J.Y., Morozowich, W. and Higuchi, W.I. (1977) Physical model approach to the design of drugs with improved intestinal absorption. In: E.B. Roche (Ed.), Design of Biopharmaceutical Properties Through Prodrugs and Analogs, American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Washington, DC, pp. 136–227.
114 Yalkowsky, S.H. and Morozowich, W. (1980) A physical chemical basis of the design of orally active prodrugs, Drug Design 9, 122–185.
115 Westergaard, H. and Dietschy, J.M. (1974) Delineation of the dimension and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine, J. Clin. Invest. 54, 718–732.
116 Wilson, F.A. and Dietschy, J.M. (1974) The intestinal unstirred layer: its surface area and effect on active transport kinetics, Biochim. Biophys. Acta 363, 112–126.
117 Charman, W.N.A., Noguchi, T. and Stella, V.J. (1986) An experimental system designed to study the in-situ lymphatic transport of lipophilic drugs in anesthetized rats, Int. J. Pharm. 33, 155–164.
118 Stella, V.J. and Charman, W.N.A. (1989) Intestinal lymphatic transport of lipophilic molecules. In: L.F. Prescott and W.S. Nimmo (Eds.), Novel Drug Delivery and Its Therapeutic Application, John Wiley, London, pp. 57–68.
119 Ueda, C.T., Lemaire, M., Gsell, G. and Nussbaumer, K. (1983) Intestinal lymphatic absorption of cyclosporin A following oral administration in an olive oil solution in rats, Biopharm. Drug Dispos. 4, 113–124.
120 Cefalu, W.T. and Pardridge, W.M. (1985) Restrictive transport of a lipid-soluble peptide (cyclosporin) through the blood-brain barrier, J. Neurochem. 45, 1954–1956.
121 Takada, K., Shibata, N., Yoshimura, H., Masuda, Y., Yoshikawa, H., Muranishi, S. and Oka, T. (1985) Promotion of the selective lymphatic delivery of cyclosporin A by lipid-surfactant mixed micelles, J. Pharmacobiodyn. 8, 320–323.
122 Takada, K., Yoshimura, H., Shibata, N., Masuda, Y., Yoshikawa, H., Muranishi, S., Yasumura, T. and Oka, T. (1986) Effect of administration route on the selective lymphatic delivery of Cyclosporin A by lipid-surfactant mixed micelles, J. Pharmacobiodyn. 9, 156–160.
123 Takada, K., Yoshimura, H., Yoshikawa, H., Muranishi, S., Yasumura, T. and Oka, T. (1986) Enhanced lymphatic delivery of cyclosporin A by solubilizers and intensified immunosuppressive activity against mice skin allograft, Pharm. Res. 3, 48–51.
124 Yoshikawa, H., Satoh, Y., Naruse, N., Takada, K. and Muranishi, S. (1985) Comparison of disappearance from blood and lymphatic delivery of human fibroblast interferon in rat by different administration routes, J. Pharmacobiodyn. 8, 206–210.
125 Van Deurs, B., Petersen, O.P. and Sandvig, K. (1989) The ways of endocytosis, Int. Rev. Cyt. 117, 131–177.
126 Eldem, T. and Speiser, P. (1989) Endocytosis and intracellular drug delivery, Acta Pharm. Technol. 35, 109–115.
127 Shepherd, V.L. (1989) Intracellular pathways and mechanisms of sorting in receptor-mediated endocytosis, TiPS 10, 458–462.
128 Mellman, I., Howe, Ch. and Helenius A. (1987) The control of membrane traffic on the endocytotic pathway, Curr. Top. Membranes Transp. 29, 255–281.
129 Silverstein, S., Steinman, R.M. and Cohn, Z.A. (1977) Endocytosis, Annu. Rev. Biochem. 46, 669–722.
130 Wall, D.A. and Maack, T. (1985) Endocytotic uptake, transport, and catabolism of proteins by epithelial cells, Am. J. Physiol. 248, C12–C20.
131 Bye, W.A., Allan, C.H. and Trier, J.S. (1984) Structure, distribution, and origin of M cells in Pey-

er's patches of mouse ileum, Gastroenterology 86, 789–801.
132 Neutra, M.R., Phillips, T.L., Mayer, E.L. and Fishkind, D.J. (1987) Transport of membrane-bound macromolecules by M cells in follicle-associated epithelium of rabbit Peyer's patch, Cell Tissue Res. 247, 537–546.
133 Munro, H.N. and Linder, M.C. (1978) Ferritin: structure, biosynthesis, and role in iron metabolism, Physiol. Rev. 58, 317–333.
134 Sanders, E. and Ashworth, E. (1961) A study of particulate intestinal absorption and hepatocellular uptake. Use of polystyrene latex particles, Exp. Cell. Res. 22, 137–145.
135 Cornell, R., Walker, A. and Isselbacher, K.J. (1971) Small intestinal absorption of horseradish peroxidase. A cytochemical study, Lab. Invest. 25, 42–48.
136 Woodley, J. and Naisbett, B. (1989) The potential of lectins as vehicles for oral drug delivery: In vitro and in vivo uptake of tomato lectin, Proc. Intern. Symp. Control. Rel. Bioact. Mater. 16, 58–59.
137 Walker, A. and Isselbacher, K.J. (1974) Uptake and transport of macromolecules by the intestine. Possible role in clinical disorders, Gastroenterology 67, 531–550.
138 Warshaw, A.L., Walker, W.A., Cornell, R. and Isselbacher, K. (1971) Small intestinal permeability to macromolecules: transmission of horseradish peroxidase into mesenteric lymph and portal blood, J. Lab. Inv. 25, 675–684.
139 Heyman, M., Ducroc, R., Desjeux, J.-F. and Morgart, J.L. (1982) Horseradish peroxidase transport across adult rabbit jejunum in vitro, Am. J. Physiol. 245, G558–G564.
140 Colony, P.C. and Neutra, M.R. (1985) Macromolecular transport in the fetal rat intestine, Gastroenterology 89, 294–306.
141 Tsujii, T., Akita, M., Katayama, K., Yamamoto, S. and Seno, S. (1984) Absorption of elastase through the jejunal mucosa of the rat. An immunocytochemical study, Histochemistry 81, 427–433.
142 Abrahamson, D.R. and Rodewald, R. (1981) Evidence for the sorting of endocytotic vesicles contents during the receptor-mediated transport of IgG across the newborn rat intestine, J. Cell Biol. 91, 270–280.
143 Rodewald, R. (1980) Distribution of immunoglobulin receptors in the small intestine of the young rat, J. Cell Biol. 85, 18–32.
144 Hasegawa, H., Nakamura, A., Watanabe, K., Brown, W.R. and Nagura, H. (1987) Intestinal uptake of IgG in suckling rats. Distinction between jejunal and ileal epithelial cells demonstrated by simultaneous ultrastructural localization of IgG and acid phosphatase, Gastroenterology 92, 186–191.
145 Patel, H. (1989) Transcytosis of drug carriers carrying peptides across epithelial barriers, Biochem. Soc. Trans. 17, 940–942.
146 Yoshikawa, H. and Muranishi, S. (1988) Absorption enhancement of cytokines into lymphatics from oral and rectal routes by fusogenic lipids, J. Interferon Res. 8, s159.
147 Yoshikawa, H., Takada, K., Satoh, Y., Naruse, N. and Muranishi, S. (1986) Development of interferon suppositories. I. Enhanced rectal absorption of human fibroblast interferon by fusogenic lipid via lymphotropic delivery in rats, Pharm. Res. 3, 116–117.
148 Yoshikawa. H., Takada, K., Muranishi, S., Satoh, Y. and Naruse, N. (1984) A method to potentiate enteral absorption of interferon and selective delivery into lymphatics, J. Pharmacobiodyn. 7, 59–62.
149 Nishihata, T., Yasui, K., Yamazaki, M. and Kamada, A. (1984) Effect of adjuvants on the rectal absorption and lymphatic uptake of pepleomycin in rats, J. Pharm. Dyn. 7, 278–285.
150 Garvey, J.F.W., Duggin, G.G., Stewart, G.J., Deane, S.A. and Little, J.M. (1985) Lymphatic absorption of cyclosporine in normal and pancreatectomized dogs, Transplant. Proc. 17, 1432–1435.
151 Vellenga, L., Mouwen, J.M.V.M., van Dijk, J.E. and Breukink, H.J. (1985) Biological and pathological aspects of the mammalian small intestinal permeabilities to macromolecules, Vet. Q. 7, 322–332.
152 Keren, D.F., Holt, P.S., Collins, H.H., Gemski, P. and Formal, S.B. (1978) The role of Peyer's patches in the local immunoglobulin A response of rabbit ileum to live bacteria, J. Immunol. 120, 1892–1896.
153 McDermott, M.R., O'Neill, M. and Bienenstock, M.J. (1980) Selective localization of lymphob-

lasts prepared from guinea pig intestinal lamina propria, J. Cell. Immunol. 51, 345–348.
154 Rosner, A.J. and Keren, D.F. (1984) Demonstration of M cells in the specialized follicle-associated epithelium overlying isolated lymphoid follicles in the gut, J. Leukocyte Biol. 35, 397–404.
155 Owen, R.L. and Ermak, T.H. (1990) Structural specializations for antigen uptake and processing in the digestive tract, Springer Semin. Immunopathol. 12, 139–152.
156 Ten Cate, C.L. (1969) Casings with 'patches of Peyer'. ΠΕΡΙ ΑΔΕΝΩΝ: from sausage to Hippokrates, Janus 56, 22–45.
157 Skinner, J.M. (1975) The immunocytes of the gastrointestinal tract. In: S.C. Truelove and M.J. Goodman (Eds.), Topics in Gastroenterology 3, Blackwell, Oxford, pp. 227.
158 Abe, K. and Ito, T. (1974) Vascular permeability in the thymus, Arch. Histol. Jap. 36, 251–264.
159 Blau, J.N. (1977) A comparative study of the microcirculation in the guinea-pig thymus, lymph nodes and Peyer's patches, Clin. Exp. Immunol. 27, 340–347.
160 Cornes, J.S. (1965) Number, size, and distribution of Peyer's patches in the human small intestine, Gut, 6, 225–233.
161 Pospischil, A. (1989) Strukturen und Funktion von Peyer'schen Platten im Darm verschiedener Tierarten, Schweiz. Arch. Tierheilk. 131, 595–603.
162 Abe, K. and Ito, T.T. (1977) A qualitative and quantitative morphologic study of Peyer's patches of the mouse, Arch. Histol. Jap. 40, 407–420.
163 Chu, R.M., Glock, R.D. and Ross, R.F. (1979) Gut-associated lymphoid tissues of young swine with emphasis on dome epithelium of aggregated lymph nodules (Peyer's patches) of the small intestine, Am. J. Vet. Res. 40, 1720–1728.
164 Carlens, O. (1928) Studien über das lymphatische Gewebe des Darmkanals bei einigen Haustieren, mit besonderer Berücksichtigung der embryonalen Entwicklung, der Mengenverhältnisse und der Altersinolution dieses Gewebes im Dünndarm des Rindes, Z. Anat. Entw. 86, 393–493.
165 Crabbé, P.A., Nash, D.R., Bazin, H., Eyssen, H. and Heremans, J.F. (1970) Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice, Lab. Invest. 22, 448–457.
166 Nagura, H., Hasegawa, H., Yoshimura, S., Aihara, K., Watanabe, K., Sawamura, S. and Ozawa, A. (1981) Comparative immunohistochemical studies on conventional and germfree rat intestinal mucosa: with special reference to microbial flora and secretory IgA (sIgA). In: S. Sasaki and A. Ozawa (Eds.), Recent Advances In Germfree Research, Tokai University Press, Tokyo, pp. 51–57.
167 Reynolds, J., Pabst, R. and Bordmann, G. (1985) Evidence for the existence of two distinct types of Peyer's patches in sheep. In: G.G.B. Klaus (Ed.), Microenvironments in the lymphoid system, Plenum Press, New York, pp. 101–109.
168 Hein, W.R., Dudler, L. and Mackay, C.R. (1989) Surface expression of differentiation antigens on lymphocytes in the ileal and jejunal Peyer's patches of lambs, Immunology 68, 365–370.
169 Sonntag, C.F. (1922) On the anatomy of the drill (*Mandrillus leucophoes*), Proc. Zool. Soc. Lond. i, 429–453.
170 Owen, R.L. and Jones, A.L. (1974) Specialized lymphoid follicle epithelial cells in the human and nonhuman primate: a possible antigen uptake site. In: O. Johari and I. Corvin (Eds.), Scanning Electron Microscopy (Part III), Proceedings of the workshop on advances in biomedical application of the SEM, IIT Research Institute Chicago, IL, pp. 697–704.
171 Rubas, W., Jezyk, N., Kos, R. and Grass, G.M. (1990) In vitro transport characteristics of Peyer's patches for actively and passively transported compounds, Proceed. Intern. Symp. Control. Rel. Bioact. Mat. 17, 309–310.
172 Faulk, W.P., McGormick, J.N., Goodman, J.R., Yoffey, J.M. and Fudenberg, H.H. (1971) Peyer's patches: Morphologic studies, Cell Immunol. 1, 500–520.
173 Pappo, J. (1989) Generation and characterization of monoclonal antibodies recognizing follicle epithelial M cells in rabbit gut-associated lymphoid tissue, Cell. Immunol. 120, 31–41.
174 Butcher, E.C., Rouse, R.V., Coffman, R.L., Nottenburg, C.N., Hardy, R.R. and Weissman, I.L. (1982) Surface phenotype of Peyer's patch germinal center cells: implication for the role of germinal centers in B cell differentiation, J. Immunol. 129, 2698–2707.
175 Hirata, I., Berrebi, G., Austin, L.L., Keren, D.F. and Dobbins, W.O. (1986) Immunohistological characterization of intraepithelial and lamina propria lymphocytes in control ileum and colon and inflammatory bowel disease, Digest. Dis. Sci. 31, 593–603.

176 Nagura, H., Tsutsumi, Y., Shimamura, K., Shioda, Y., Hasegawa, H. and Tamaoiki, K. (1984) Immunohistochemical observation of human solitary lymphoid follicles, Dig. Organ Immunol. 12, 89–93.
177 Guy-Grand, D., Griscelli, C. and Vassalli, P. (1974) The gut associated lymphoid system: nature and properties of the large dlvlding cells, Eur. J. Immunol. 4, 435–443.
178 McWilliams, M., Lamm, M.E. and Phillips-Quagliata, J.A. (1974) Surface and intracellular markers of mouse mesenteric and peripheral lymph node and Peyer's patch cells, J. Immunol. 113, 1326–1333.
179 Roelants, G.E., Loor, F., Von Boehmer, H., Sprent, J., Hagg, L.B., Mayor, K.S. and Ryden, A. (1975) Five types of lymphocytes characterized by double immunofluorescence and electrophoretic mobility. Organ distribution in normal and nude mice, Eur. J. Immunol. 5, 127–131.
180 Ermak, T.H., Steger, H.J. and Pappo, J. (1990) Phenotypically distinct subpopulations of T cells in domes and M-cell pockets of rabbit gut-associated lymphoid tissue, Immunology 71, 530–537.
181 MacDonald, T.T., Spencer, J., Viney, J.L., Williams, C.B. and Walker-Smith, J.A. (1987) Selective biopsy of human Peyer's patches during ileal endoscopy, Gastroenterology 93, 1356–1362.
182 Cebra, J.J., Crandall, C.A., Gearhart, P.J., Robertson, S.M., Seng, J.T. and Watson, P.M. (1979) Cellular events concerned with the initiation, expression and control of the mucosal immune response. In: P. Ogra and D. Dayton (Eds.), Immunology of Breast Milk, Raven Press, New York, pp. 1–8.
183 Gearhart, P.J. and Cebra, J.J. (1979) Differentiated B lymphocytes. Potential to express particular antibody variable and constant regions depends on site of lymphoid tissue and antlgen load, J. Exp. Med. 149, 216–227.
184 Kawanishi, H., Ozato, K. and Strober, W. (1985) The proliferative response of cloned Peyer's patch switch T cells to syngeneic and allogeneic stimuli, J. Immunol. 134, 3586–3591.
185 Kiyono, H., McGhee, J.R., Mosteller, L.M., Eldrige, J.M., Koopman, W.J., Kearney, J.F. and Michalek, S.M. (1982) Murine Peyer's patch T cell clones. Characterization of antigen-specific helper T cells for immunoglobulin A responses, J. Exp. Med. 156, 1115–1130.
186 Pabst, R. (1988) The spleen in lymphocyte migration, Immunol. Today 9, 43–45.
187 Duijvestijn A. and Hamann A. (1989) Mechanisms and regulation of lymphocyte migration, Immunol. Today 10, 23–28.
188 Seifert, J. and Sass, W. (1988) Nachweis der Resorption von Makromolekülen und Partikeln, Acta Pharm. Technol. 34, 55–62.
189 Carey, G.D., Chin, Y.-H. and Woodruff, J.J. (1981) Lymphocyte recognition of lymph node high endothelium. III. Enhancement by a component of thoracic duct lymph, J. Immunol. 127, 976–979.
190 Gallatin, W.M., Weissmann, I.L. and Butcher, E.C. (1983) A cell-surface molecule involved in organ-specific homing of lymphocytes, Nature 304, 30–34.
191 Schmitz, M., Nuñez, D. and Butcher, E. (1988) Selective recognition of mucosal lymphoid high endothelium by gut intraepithelial leukocytes, Gastroenterology 94, 576–581.
192 Guy-Grand, D., Grlscelli, C. and Vassalli, P. (1978) The mouse gut T lymphocyte, a novel type of T cell. Nature, origln and traffic in mice in normal and graft-versus-host conditions, J. Exp. Med. 148, 1661–1677.
193 Kagnoff, M.F. (1987) Immunology of the digestive system. In: L.R. Johnson, J. Christen, M.J. Jackson, E.D. Jacobson and J.H. Walsh (Eds.), Physiology of the Gastrointestinal Tract, Raven Press, New York, Vol. 2, pp. 1699–1728.
194 Stoolman, L.M. and Ebing, H. (1989) Adhesion molecules of cultured hematopoletic malignancles. A calcium-dependent lectin ls the principle mediator of binding to the high endothelial venule of lymph nodes, J. Clin. Invest. 84, 1196–1205.
195 Pober, J.S. and Cotran, R.S. (1990) Cytokines and endothelial cell biology, Physiol. Rev. 70, 427–451.
196 Rose, M.L., Parrott, D.M.V. and Bruce, R.G. (1976) Mlgration of lymphoblasts to the small intestine. II. Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse, Cell Immunol. 27, 36–46.
197 Rosen, S.D., Singer, M.S. and Yednock, T.A. (1985) Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation, Science 228, 1005–1007.
198 Pabst, R. and Reynolds, J.D. (1987) Peyer's patches export lymphocytes throughout the lymphoid

system in sheep, J. Immunol. 139, 3981–3985.
199 Binns, R.M., Pabst, R. and Licence, S.T. (1985) Lymphocyte emigration from lymph nodes by blood in the pig and efferent lymph in the sheep, Immunology 54, 105–111.
200 Miura, S., Sekizuka, E., Nagata, H., Oshio, C., Minamitani, H., Suematsu, M., Suzuki, M., Hamada, Y., Kobayashi, K., Asakura, H. and Tsuchiya, M. (1987) Increased lymphocyte transport by lipid absorption in rat mesenteric lymphatics, Am. J. Physiol. 253, G596–G600.
201 Mahida, Y.R., Patel, S. and Jewell, D.P. (1989) Mononuclear phagocyte system of human Peyer's patches: an immunohistochemical study using monoclonal antibodies, Clin. Exp. Immunol. 75, 82–86.
202 Momotani, E., Whipple, D.L., Thiermann, A.B. and Cheville, N.F. (1988) Role of M cells and macrophages in the entrance of Mycobacterium paratuberculosis into domes of ileal Peyer's patches in calves, Vet. Pathol. 25, 131–137.
203 Owen, R.L., Allen, C.L. and Stevens, D.P. (1981) Phagocytosis of Giardia muris by macrophages in Peyer's patch epithelium in mice, Infection Immunity 33, 591–601.
204 Pappo, J. (1989) Generation and characterization of monoclonal antibodies recognizing follicle epithelial M cells in rabbit gut-associated lymphoid tissues, Cell. Immunol. 120, 31–41.
205 Hayakawa, E. and Lee, V.H.L. (1987) Peyer's patches as a possible site of oral peptide delivery: Nature and magnitude of proteolytic barrier, F.I.P., 47th International Congress of Pharmaceutical Sciences of F.I.P., pp. 235 (abstr.).
206 McClugage, S.G., Low, F.N. and Zimny, M.L. (1986) Porosity of the basement membrane overlying Peyer's patches in rats and monkeys, Gastroenterology 91, 1128–1133.
207 McGlugage, S. and Low, F. (1984) Microdissection by ultrasonication: porosity of the intestinal epithelial basal lamina, Am. J. Anat. 171, 207–216.
208 Pappo, J., Steger, H.J. and Owen, R.L. (1988) Differential adherence of epithelium overlying gut-associated lymphoid tissue, Lab. Invest. 58, 692–697.
209 Inman, L.R. and Cantey, J.R. (1983) Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer's patch in *Escherichia coli* diarrhea in the rabbit, J. Clin. Invest. 71, 1–8.
210 Bockman, D.E. and Cooper, M.D. (1973) Pinocytosis by epithelium associated with lymphoid follicles in the bursa of fabricius, appendix, and Peyer's patches. An electron microscopic study, Am. J. Anat. 136, 455–478.
211 Owen, R.L. and Jones, A.L. (1974) Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles, Gastroenterology 66, 189–203.
212 Owen, R.L. (1977) Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study, Gastroenterology 72, 440–451.
213 Sass, W., Dreyer, H.P., Böckeler, W., Mamelmann, H. and Seifert, H. (1987) Prinzipien der Partikelresorption im Magen-Darm-Trakt, Z. Gastroenterol. 25, 306–315.
214 Mayrhofer, G., Pugh, C.W. and Barclay A.N. (1983) The distribution, ontogeny and origin in the rat of Ia-positive cells with dendritic morphology and of Ia antigen epithelia, with special reference to the intestine, Eur. J. Immunol. 13, 112–122.
215 Dukes, C. and Bussey, H.J.R. (1926) The number of lymphoid follicles of human large intestine, J. Pathol. Bacteriol. 29, 111–117.
216 Egberts, H.J.A., Brinkhoff, M.G.M., Mouwen, J.M.V.M., Van Dijk, J.E. and Koninkx, J.F.G. (1985) Biology and pathology of the intestinal M-cell. A review, Vet. Q. 7, 333–336.
217 Wolf, J.L. and Bye, W.A. (1984) The membranous epithelial (M) cell and the mucosal immune system, Annu. Rev. Med. 35, 95–112.
218 Bockman, D.E. and Cooper, M.D. (1971) Fine structural analysis of pinocytosis in lymphoid follicle associated epithelium in chick bursa and rabbit appendix, Fed. Proc. 30, 511–516.
219 Owen, R.L. and Bhalla, D.K. (1983) Cytochemical analysis of alkaline phosphatase and esterase activities and of lectin-binding and anionic sites in rat and mouse Peyer's patch M cells, Am. J. Anat. 168, 199–212.
220 Smith, M.W. and Peacock, M.A. (1980) 'M' cell distribution in follicle-associated epithelium of mouse Peyer's patch, Am. J. Anat. 159, 167–175.
221 Torres-Medina, A. (1981) Morphologic characteristics of the epithelial surface of aggregated

lymphoid follicles (Peyer's patches) in the small intestine of newborn gnotobiotic calves and pigs, Am. J. Vet. Res. 42, 232–236.
222 Landsverk, T. (1981) The epithelium covering Peyer's patches in young milk-fed calves. An ultrastructural and enzyme histochemical investigation, Acta Vet. Scand. 22, 198–210.
223 Owen, R.L. and Nemanic, P. (1978) Antigen processing structures of the mammalian intestinal tract: An SEM study of lymphoepithelial organs, Scanning Electron Microscopy 11, 367–378.
224 Owen, R.L. and Johnes, A.L. (1974) Specialized lymphoid follicle epithelial cells in the human and non human primate: A possible antigen uptake site. In: Scanning Electron Microscopy (Part III). Proceedings of the workshop on advances in biochemical applications of the SEM Chicago, IIT Research Institute, pp. 697–704.
225 Befus, A.D., Johnston, G.A., Leslie, G.A. and Bienenstock, J. (1980) Gut-associated lymphoid tissue In the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches, J. Immunol. 125, 2626–2631.
226 Sneller, M.C. and Strober, W. (1986) M cells and host defense, J. Infect. Dis. 154, 737–741.
227 Kanou, T. (1984) Morphological study of microfold cells of intestinal lymphoid follicles in Peyer's patches, Kawasaki Med. J. 10, 181–189.
228 Ho, N.F.H., Day, J.S., Barsuhn, C.L., Burton, P.S. and Raub, T.J. (1990) Biophysical model approach to mechanistic transepithelial studies of peptides, J. Controlled Rel. 11, 3–24.
229 Smith, M.W., James, P.S. and Tivey, D.R. (1987) M cell numbers increase after transfer of SPF mice to a normal animal house environment, Am. J. Pathol. 128, 385–389.
230 Madara, J.L., Bye, W.A. and Trier, J.S. (1984) Structural features of and cholesterol distribution in M-cell membranes in guinea pig, rat, and mouse Peyer's patches, Gastroenterology 87, 1091–1103.
231 Sicinski, P. (1988) Morphometric characteristics of the luminal surface of human intestinal M cells, J. Histochem. 36, 876 (abstr.).
232 Sicinski, P., Rowinski, J., Warchol, J.B. and Bem, W. (1986) Morphometric evidence against lymphocyte-induced differentiation of M cells from absorptive cells in mouse Peyer's patches, Gastroenterology 90, 609–615.
233 Bhalla, D.K. and Owen, R.L. (1982) Cell renewal and migration in lymphoid follicles of Peyer's patches and cecum – An autoradiographic study in mice, Gastroenterology 82, 232–242.
234 Wilders, M.M., Drexhage, H.A., Weltevreden, E.F., Mullink, H., Duijvestijn, A and Meuwissen, S.G.M. (1983) Large mononuclear la-positive veiled cells in Peyer's patches. I. Isolation and characterization in rat, guinea-pig and pig, Immunology 48, 453–460.
235 Wilders, M.M., Sminia, T., Plesch, B.E.C., Drexhage, H.A., Weltevreden, E.F. and Meuwissen, S.G.M. (1983) Large mononuclear Ia-positive veiled cells in Peyer's patches. II. Localization in rat Peyer's patches, Immunology 48, 461–467.
236 Nagura, H. and Sumi, Y. (1986) Mucosal defense mechanism of the gut – Role of the mucosal immune system, Saisin Igaku 41, 2760–2766.
237 Walker, W.A., Cornell, R., Davenport, L.M. and Isselbacher, K.J. (1972) Macromolecular absorption mechanism of horseradish peroxidase uptake and transport in adult and neonatal rat intestine, J. Cell. Biol. 54, 195–205.
238 Heyman, H., Ducroc, R., Desjeux, J.-F. and Morgat, J.L. (1982) Horseradish peroxidase transport across adult rabbit jejunum in vitro, Am. J. Physiol. 242, G558–G564.
239 Keljo, D.J. and Hamilton, J.R. (1983) Quantitative determination of macromolecular transport rate across intestinal Peyer's patches, Am. J. Physiol. 244, G637–G644.
240 Owen, R.L., Apple, R.T. and Bhalla, D.K. (1986) Morphometric and cytochemical analysis of lysosomes in rat Peyer's patch follicle epithelium: their reduction in volume fraction and acid phosphatase content in M cells compared to adjacent enterocytes, Anat. Rec. 216, 521–521.
241 Stiglmair-Herb, M.T., Pospischil, A., Hess, R.G., Bachmann, P.A. and Baljer, G. (1986) Enzyme histochemistry of the small intestinal mucosa in experimental infections of calves with rotavirus and enterotoxigenic *Escherichia coli*, Vet. Pathol. 23, 125–131.
242 Neutra, M.R., Guerina, N.G., Hall, T.L. and Nicolson, G.L. (1982) Transport of membrane-bound macromolecules by M cells in rabbit intestine, Gastroenterology 82, 1137.
243 Roy, M.J. and Varvayanis, M. (1987) Development of dome epithelium in gut-associated lymphoid tissues: Association of IgA with M cells, Cell Tissue Res. 248, 645–651.

244 Kawanishi, H., Saltzman, L. and Strober, W. (1983) Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. II. Terminal differentiation of postswitch sIgA-bearing Peyer's patch B cells, J. Exp. Med. 158, 649–669.
245 Weltzin, R., Kraehenbuhl, J.P. and Neutra, M.R. (1987) Mononclonal IgA adheres to luminal surfaces of M cells in Peyer's patch epithelium, J. Cell Biol. 105, 234a.
246 Weltzin, R., Lucia-Jandris, P., Michetti, P., Fields, B.N., Kraehenbuhl, J.P. and Neutra, M.R. (1989) Binding and transepithelial transport of immunoglobulins by intestinal M cells: demonstration using monoclonal IgA antibodies against enteric viral proteins, J. Cell. Biol. 108, 1673–1685.
247 Nagura, H. and Sumi, Y. (1988) Immunological function of the gut-role of the mucosal immune system, Toxicol. Pathol. 16, 154–164.
248 Abuchowski, A. and Davis, F.F. (1981) Soluble polymer-enzyme adducts. In: J.C. Holcenberg and J. Roberts (Eds.), Enzymes as Drugs, John Wiley, New York, pp. 367–383.
249 Pappo, J. and Owen, R.L. (1988) Absence of secretory component expression by epithelial cells overlying rabbit gut-associated lymphoid tissue, Gastroenterology 95, 1173–1177.
250 Solari, R. and Kraehenbuhl, J.P. (1985) The biosynthesis of secretory component and its role in the transepithelial transport of IgA dimer, Immunol. Today 6, 17–20.
251 Solari, R., Kuhn, L. and Kraehenbuhl, J.P. (1985) Antibodies recognizing different domains of the polymeric immunoglobulin receptor, J. Biol. Chem. 260, 1141–1145.
252 Isolauri, E., Gotteland, M., Heyman, M., Pochart, P. and Desjeux, J.F. (1990) Antigen absorption in rabbit bacterial diarrhea (RDEC-1). In vitro modifications in ileum and Peyer's patches, Digestive Diseases and Sciences 35, 360–366.
253 Sicinski, P., Rowinski, J., Warcholz, J.B., Jarzabek, Z., Gut, W., Szczygiel, B., Bielechi, K. and Koch, G. (1990) Poliovirus type 1 enters the human host through intestinal M cells, Gastroenterology 98, 56–58.
254 Wassef, J., Keren, D.F. and Mailloux, J.L. (1989) Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis, Infect. Immun. 57, 858–863.
255 Kantele, A., Arvilommi, A. and Jokinen, I. (1986) Specific immunoglobulin-secreting human blood cells after peroral vaccination against Salmonella typhi, J. Inf. Dis. 153, 1126–1131.
256 Ussing, H.H. and Zehran, K. (1951) Active transport of sodium as the source of electric current in the short-circuited isolated frog skin, Acta Physiol. Scand. 23, 110–127.
257 Grass, G.M. and Sweetana, S.A. (1988) In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell, Pharm. Res. 5, 372–376.
258 Keljo, D.J., Butler, D.G. and Hamilton, J.R. (1985) Altered jejunal permeability to macromolecules during viral enteritis in the piglet, Gastroenterology 88, 998–1004.
259 Ducroc, R., Heyman, M., Beaufrere, B., Morgat, J.L. and Desjeux, J.F. (1983) Horseradish peroxidase transport across rabbit jejunum and Peyer's patches in vitro, Am. J. Physiol. 245, G54–G58.
260 Beahon S.J. and Woodley J.F. (1984) The effect of oral immunization on the uptake of macromolecules by adult rat intestinal columnar epithelium and Peyer's patch tissue in vitro, Biochem. Soc. Trans. 12, 1087.
261 Beahon, S.J. and Woodley, J.F. (1984) The uptake of macromolecules by adult rat intestinal columnar epithelium and Peyer's patch tissue in vitro, Blochem. Soc. Trans. 12, 1088.
262 Heyman, M., Bonfils, A., Fortier, M., Crain-Denoyelle, A.M., Smets, P. and Desjeux, J.F. (1987) Intestinal absorption of RU 41740, an immunomodulating compound extracted from Klebsiella pneumoniae, across duodenal epithelium and Peyer's patches of the rabbit, Int. J. Pharm. 37, 33–39.
263 LeFevre, M.E., Hancock, D.C. and Joel, D.D. (1980) The intestinal barrier to large particulates in mice, J. Toxicol. Environ. Health 6, 691–704.
264 Gilley, R.M., Eldrige, J.H., Opitz, J.L., Hanna, L.K., Staas, J.K. and Tice, T.R. (1988) Development of secretory and systemic immunity following oral administration of microencapsulated antigens, Proceed. Intern. Symp. Control. Rel. Bioact. Mat. 15, 123–124.
265 Joel, D.D., Laissue, J.L. and LeFevre, M.E. (1978) Distribution and fate of ingested carbon particles in mice, J. Reticuloendothel. Soc. 24, 477–487.
266 LeFevre, M.E., Bocci, A.M. and Joel, D.D. (1989) Intestinal uptake of fluorescent microspheres in young and aged mice, Proc. Soc. Exp. Biol. Med. 190, 23–27.

in young and aged mice, Proc. Soc. Exp. Biol. Med. 190, 23–27.
267 Pappo, J. and Ermak, T.H. (1989) Uptake and translocation of fluorescent latex particles by rabbit Peyer's patch follicle epithelium: a quantitative model for M cell uptake, Clin. Exp. Immunol. 76, 144–148.
268 Eldridge, J.H., Gilley, R.M., Staas, J.K., Moldoveanu, Z., Meulbroek, J.A. and Tice, T.R. (1989) Biodegradable microspheres: vaccine delivery system for oral immunization. In: Current Topics in Microbiology and Immunology, Springer Verlag, Berlin, Vol. 146, pp. 59–66.
269 Ebel, J.P. (1990) A method for quantifying particle absorption from the small intestine of the mouse, Pharm. Res. 7, 848–851.
270 Tice, T.R., Staas, J.K., Gilley, R.M. and Eldridge, J.H. (1988) Method and formulation for orally administering bioactive agents to and through the Peyer's patches, EP O 266 119 A2.
271 Payne, J.M., Sansom, Garner, R.J., Thomson, A.R. and Miles, B.J. (1960) Uptake of small resin particles (1–5 μm diameter) by the alimentary canal of the calf, Nature 188, 586–587.
272 Eldridge, J.H., Hammond, Ch.J., Meulbroek, J.A., Staas, J.K., Gilley, R.M. and Tice, T.R. (1990) Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches, J. Controlled. Release 11, 205–214.
273 LeFevre, M.E. and Joel, D.D. (1984) Peyer's patch epithelium: an imperfect barrier. In: C.M. Schiller (Ed.), Intestinal Toxicology, Raven Press, New York, pp. 45–56.
274 LeFevre, M.E., Warren, J.B. and Joel, D.D. (1985) Particles and macrophages in murine Peyer's patches, Exp. Cell. Biol. 53, 121–129.
275 Jani, P., Halbert, G.W., Langridge, J. and Florence, A.T. (1989) The Uptake and Translocation of Latex Nanospheres and Microspheres after Oral Administration to Rats, J. Pharm. Pharmacol. 41, 809–812.
276 Jani, P., Halbert, G.W., Langridge, J. and Florence, A.T. (1990) Nanoparticle Uptake by the Rat Gastrointestinal Mucosa: Quantitation and Particle Size Dependency, J. Pharm. Pharmacol. 42, 821–826.
277 De Keyser, J.-L., Poupaert, J.H. and Dumont, P. (1991) Poly(diethylmethyldenemalonate) nanoparticles as a potential drug carrier: preparation, distribution and elimination after intravenous and peroral administration to mice, J. Pharm. Sci. 80, 67–70.
278 Illum, L. and Davis, S.S. (1984) The organ uptake of intravenously administered colloidal particles can be altered using non-ionic surfactant (Poloxamer 338), FEBS Lett. 167, 79–82.
279 Alpar, H.O., Field, W.N., Hyde, R. and Lewis, D.A. (1989) The transport of microspheres from the gastro-intestinal tract to inflammatory air pouches in the rat, J. Pharm. Pharmacol 41, 194–196.
280 LeFevre, M.E., Olivo, R., Vanderhoff, J.W. and Joel, D.D. (1978) Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes, Proc. Soc. Exp. Biol. Med. 159, 298–302.
281 LeFevre, M.E., Joel, D.D. and Schidlovsky, G. (1985) Retention of ingested latex particles in Peyer's patches of germfree and conventional mice, Proc. Soc. Exp. Biol. Med. 179, 522–528.
282 Schmidt, P.C. and Michaelis, J. (1990) Liposomen zur oralen Anwendung. 1. Mitteilung: Literaturüberblick zur Stabilität und Resorption von Liposomen in vitro/in vivo und zu Ergebnissen mit in vivo getesteten Arzneistoffen, Pharm. Z. Wiss. 135, 125–134.
283 Michalek, S.M., Childers, N.K., Katz, J. and Curtiss, R. (1987) Oral vaccines and the secretory immune system, Clin. Immun. Lett. 8, 158–159.
284 Childers, N.K., Denys, F. and Michalek, S.M. (1988) Uptake of gold-labelled liposomes by rat Peyer's patch M-cells, Annu. Meet. Am. Soc. Microbiol. 88, 124.
285 Michalek, S.M., Childers, N.K., Katz, J., Denys, F.R., Berry, A.K., Eldridge, J.H., McGhee, J.R. and Curtiss, R. (1989) Liposomes as oral adjuvants. In: J. Mestecky and J.R. McGhee (Eds.), New Strategies for Oral Immunization, Current Topics in Microbiology and Immunology, Vol. 146, pp. 51–58.
286 Rhalem, A., Bekhti, K., Bourdleu, Ch., Luffau, G. and Péry, P. (1989) Vaccination de la souris contre le coccidiose murine par ingestion de protéines de surface d'Eimiria falciformis incorporeés dans des liposomes, C. R. Acad. Sci. Paris, Vol. 309, Série III, pp. 19–23.
287 O'Hagan, D.T. and Illum, L. (1990) Absorption of peptides and proteins from the respiratory tract and the potential for development of locally administered vaccine, Crit. Rev. Ther. Drug Carrier Syst. 7, 35–97.

288 Patel, H.M., Tuzél, N.S. and Stevenson, R.W. (1985) Intracellular digestion of saturated and unsaturated phospholipid liposomes by mucosa cells. Possible mechanism of transport of liposomally entrapped macromolecules across the isolated vascularly perfused rabbit ileum, Biochim. Biophys. Acta 839, 40–49.
289 Wolf, J.L., Rubin, D.H., Finberg, R., Kauffman, R.S., Sharpe, A.H., Trier, J.S. and Fields, B. N. (1981) Intestinal M cells: a pathway for entry of reovirus into host, Science 212, 471–472.
290 Wolf, J.L., Kauffman, R.S., Finberg, R., Dambrauska, R., Fields, B.N. and Trier, J.S. (1983) Derminants of reovirus interaction with the intestinal M cells and absorptive cells of murine intestine, Gastroenterology 85, 291–300.
291 Wolf, J.L., Dambrauskas, R., Sharpe, A.H. and Trier, J.S. (1987) Adherence to and penetration of the intestinal epithelium by reovirus type 1 in neonatal mice, Gastroenterology 92, 82–91.
292 Bassel-Duby, R., Jayasuriya, A., Chatterjee, D., Sonenberg, N., Maizel, J.V. and Fields, B.N. (1985) Sequence of reovirus haemagglutinin predicts a coiled-coil structure, Nature 315, 421–423.
293 Furlong, D.B., Nibert, M.L. and Fields, B.N.J. (1988) Sigma 1 protein of mammalian reovirus extends from the surfaces of viral particles, Virology 62, 246–256.
294 Lee, P.W., Hayes, E.C. and Joklik, W.K. (1981) Protein 1 is the reovirus cell attachment protein, Virology 108, 156–163.
295 Nagata, L., Masri, S.A., Mah, C.W. and Lee, P.W. (1984) Molecular cloning and sequencing of the reovirus (serotype 3) S1 gene which encodes the viral cell attachment protein 1, Nucleic Acid Res. 12, 8699–8710.
296 Rubas, W., Banerjea, A.C., Gallati, H., Speiser, P.P. and Joklik, W.K. (1990) Incorporation of the reovirus M cell attachment protein into small unilamellar vesicles: incorporation efficiency and binding capability to L929 cells in vitro, J. Microencaps. 7, 385–395.
297 Owen, R.L., Pierce, N.F., Apple, R.T. and Cary Jr., W.C. (1986) M cell transport of vibrio cholerea from the intestinal lumen into Peyer's patches: a mechanism for antigen sampling and for microbial transepithelial migration, J. Infect. Dis. 153, 1108–1118.
298 Walker, R.I., Schmauder-Chock. E.A., Parker, J.L. and Burr, D. (1988) Selective association and transport of campylobacter jejuni through M cells of rabbit Peyer's patches, Can. J. Microbiol. 34, 1142–1147.
299 Hackett, J., Kotlarski, I., Mathan, V., Francki, K. and Rowley, D. (1986) The colonization of Peyer's patches by a strain of salmonella typhimurium cured of the cryptic plasmid, J. Inf. Dis. 153, 1119–1125.
300 Strober, W. and Jacobs, D. (1985) Cellular differentiation, migration, and function in the mucosal immune system. In: J.I. Gallin and A.S. Fauci (Eds.), Mucosal Immunity, Raven Press, New York, pp. 1–30.
301 Gebbers, J.-O. and Laissue, J.A. (1989) Immunologic structures and functions of the gut, Schweiz. Arch. Tierheilk. 131, 221–238.
302 HogenEsch, H., Housman, J.M. and Felsburg, P.J. (1987) Canine Peyer's patches: macroscopic, light microscopic, scanning electron microscopic and immunohistochemical investigations, Adv. Exp. Med. Biol. 216A, 249–256.
303 Kelsall, M.A. (1946) Number of Peyer's patches in mice belonging to high and low mammary tumor strains, Proc. Soc. Exp. Biol. Med. 61, 423–424.
304 Sackmann, W. (1977) Anzahl und Lokalization der Peyerschen Platten in Dünndarm des Kaninchens (*Oryctolagus cuniculus*), Acta Anat. 97, 109–113.
305 Unthank, J.L. and Bohlen, H.G. (1988) Lymphatic pathways and role of valves in lymph propulsion from small intestine, Am. J. Physiol. 254, G389–G398.
306 Charman, W.N. and Stella, V.J. (1991) Transport of lipophilic molecules by the intestinal lymphatic system, Adv. Drug Dellv. Rev. 7, 1–14.